Unwinding the Mystery of Copper Nanoparticles Using Pteris Plant Extract & It's Anti-microbial Activity in Green Chemistry

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This is to certify that Akash Singh (Registration ID: 230020461411; Enrollment ID: 2321210002046), a student of M.Sc. in Biotechnology, has successfully completed his final semester dissertation work under my guidance, Dr. Joy Sarkar, Associate Professor of Botany, at Dinabandhu Andrews College, Kolkata.

The dissertation work was carried out as a part of the academic requirement for the partial fulfillment of the degree of Master of Science in Biotechnology under the School of Health Science and Translational Research, Sister Nivedita University, Kolkata.

The research project entitled *"Unwinding the Mystery of Copper Nanoparticles using Pteris Plant Extract & it's Anti-microbial Activity in Green Chemistry"* was conducted during the period January 2025 to June 2025, and the student has shown sincere dedication and scientific aptitude throughout the course of the work.

I wish the student every success in future academic and professional endeavors.

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DECLARATION BY THE STUDENT

I hereby declare that the work which is being presented in this dissertation entitled "Unwinding the Mystery of Copper Nanoparticles Using Pteris Plant Extract & It's Anti-microbial Activity in Green Chemistry" in partial fulfilment of the requirements for the award of the degree of Masters of Science in Biotechnology is an authentic record of my own work carried out during the period from to under the supervision of Dr. Joy Sarkar Department of Botany, Dinabandhu Andrews College (Calcutta University), Associate Professor & IQAC co-ordinator.

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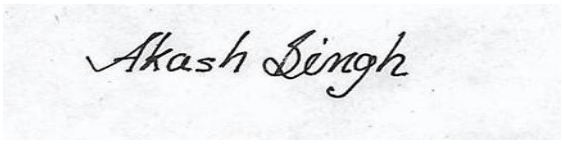

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Abbreviations

- **Cu/CuO-NPs** – Copper/Copper-oxide Nanoparticles
- **FTIR** – Fourier Transform Infrared Spectroscopy
- **DLS** – Dynamic Light Scattering
- **XRD** – X-ray Diffraction
- **UV-Vis** – Ultraviolet-Visible Spectroscopy

Abstract

Because of Copper Nanoparticle’s unique physicochemical properties that distinguish them from their bulk counterparts, nanoparticles are helpful in a range of biological and commercial applications. The primary focus of this work is the environmentally friendly and sustainable green synthesis of copper/copper oxide nanoparticles (Cu/Cu-O-NPs) using Pteris plant extract, which avoids the use of dangerous chemicals. The phytochemicals in Pteris extract acted as stabilising and reducing agents, reducing copper sulphate ($CuSO_4$,$5H_2O$) and facilitating biosynthesis. The synthesis of Cu/Cu-O-NPs was confirmed by means of FTIR analysis, XRD, DLS, and UV-Vis spectroscopy. They displayed their crystalline structure, size distribution, surface plasmon resonance, and interactions between functional groups.

The antibacterial activity of Cu/Cu-O-NPs against Escherichia coli and Bacillus subtilis was assessed using agar diffusion and broth microdilution experiments. The results demonstrated strong dose-dependent antibacterial effects, with larger zones of inhibition appearing at higher nanoparticle concentrations. Intracellular leakage, membrane rupture, and oxidative stress induced by reactive oxygen species (ROS) were likely the causes of bacterial inhibition. Furthermore, the ability of the nanoparticles to compromise bacterial integrity was confirmed by absorbance measurements at 260 and 280 nm, which confirmed the leaking of proteins and DNA.

This work highlights the need for green synthesis to create bioactive copper nanoparticles with enhanced antibacterial properties that could be useful in agricultural, medical, and environmental applications. Additional research could look at synthesis parameter optimisation, cytotoxicity, and antifungal efficacy for broader applications. The environmentally safe synthesis of Cu/Cu-O-NPs using Pteris extract is an intriguing direction in sustainable nanotechnology.

Key Words: Copper/Copper-nanoparticle; Green synthesis; UV- visible spectroscopy; DLS; XRD; FTIR; Antimicrobial activity

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Chapter-1

Introduction

Nanoparticles are discrete assemblies of atoms at the nanometre (10-9 m) scale. As a result, their dimensions are in the range of 10–10 m for ions and macroscopic solids. The particles are interesting because of their small number of atoms.

Adequate to significantly change the particle's atomic, electronic, and magnetic structures, as well as its chemical and physical characteristics and reactivity when compared to the bulk material, with a significant percentage of them at or near surfaces. Nanoparticles themselves may have distinct surfaces. End particles may have nuclear planes or clusters that are rare or not present on the surfaces of the bulk mineral. These and other size-related factors will change phase stability and reaction kinetics.[1]

What makes a nanoparticle unique? The characteristics of each material must be taken into consideration when defining size ranges for molecules, nanoparticles, and macroscopic solids. However, a useful upper limit is provided by the size at which one of the properties of a nanoparticle deviates from the value for the equivalent bulk material by a factor much larger than the error of the measuring method (a few percent). In reality, a particle will probably differ sufficiently to be called a "nanoparticle" if its diameter is less than a few tens of nanometres, and perhaps less than a fraction of a micron.[1]

Which natural systems contain nanoparticles? Where do they begin? As a distinct state of matter deserving of a book in the RIMG series, why should we treat them differently? This chapter will (1) outline the types of solids that are commonly found as nanoparticles in natural systems; (2) outline a subset of the inorganic and biological processes that produce nanoparticles in the environment; (3) explain how the stability and reactivity of nanoparticles are affected by their particle size; and (4) discuss some possible avenues for further research on nanoparticles in geological systems in order to provide a basis for thinking about the role of nanoparticles in natural processes.[1]

There are nanoparticles everywhere, or nearly everywhere! Mineralogists who work with environmental samples have become used to this. In fact, the discipline of clay mineralogy is quite developed, with "clays" being defined as particles smaller than 2 $\propto$ μm rather than layer silicates. However, there are plenty of unresolved issues surrounding size-related effects, and characterising the smallest of these particles presents significant obstacles. Biochemists are also knowledgeable in nanoparticles, which are found in key biological molecules like cells and, at the lowest size, operate as active centres of enzymes (such as [Fe4S4](Sγ Cys)4 clusters). The origin of molecules necessary for life (such proteins) and biological function and evolution may be explained by fresh insights into the size-dependent properties of tiny particles and cluster formation.[1]

	Nanoparticles	*Nanomaterials*
ISO	A particle with a diameter of 1-100nm	--
ASTM	A thin particle that ranges in length from1-100nm in two or three locations	--
NIOSH	A fibre that spans 1-100nm range	--
SCCP	The nanoscale range includes at least one side	Substance whose interior structure or atleast one side is nanoscale.
BSI	Every diameter falls within the nanoscale range	Material with an internal structure or atleast one side in the nanoscale.
*BA**u**A*	Every diameter falls with in the spectrum of nanoscales	Material made out of a nanosubstance or nano structure. [2]

Table 1.1 lists the classifications of nanomaterials and nanoparticles presented by different organisations: International Organization for Standardization (ISO), American Society of Testing and Materials (ASTM), National Institute of Occupational Safety and Health (NIOSH), Scientifi c Committee on Consumer Products (SCCP), British Standards Institution (BSI), and Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (BAuA)

In general, nanoparticles are between 1 and 100 nm in size. Metallic nanoparticles have different physical and chemical properties from bulk metals, which can be advantageous in a variety of industrial applications. Lower melting points, greater specific surface areas, particular mechanical strengths, particular optical qualities, and particular magnetisations are some of these traits. However, the specific application has a significant impact on the definition and perception of a nanoparticle. In this regard, Table 1.1 compiles the definitions of nanoparticles and nanomaterials offered by various organisations.[2]

Figure 1.1 shows pictures of the well-known Lycurgus cup, which changes colour depending on whether it is lit from the inside (b) or the outside (a). Visit the British Museum's website [2] for more information.

The optical property of a nanoparticle is one of its main attractions and characteristics. For example, a 20-nm gold nanoparticle has a characteristic wine-red colour. The colour of a silver nanoparticle is greyish yellow. Platinum and palladium nanoparticles are black. Not surprisingly, the optical properties of nanoparticles have been exploited in sculptures and paintings since before the fourth century AD. The most famous example is the Lycurgus cup. The only intact historical example of dichroic glass—a particularly odd type of glass that changes colour when exposed to light—is this one cup. The opaque green cup turns a brilliant translucent red when illuminated from within.A tiny quantity of tiny (roughly 70 nm) metal crystals of Ag and Au in a roughly molar ratio of 14:1 give the glass its extraordinary optical properties and give the Lycurgus Cup its distinctive colour display. The reader may be surprised to learn that the cup is currently in the British Museum.[2]

1.1. Green Synthesis of Cu/CuO by plant extracts:-

The biosynthesis of nanoparticles, which includes bacteria, fungi, plants, actinomycetes, and other organisms, is known as "green synthesis" and is based on the widely accepted "green chemistry" concept. The goal is to create clean and environmentally friendly nanoparticles. An environmentally friendly method of producing nanoparticles with unique properties is the biosynthesis of nanoparticles using the organisms already discussed. In these syntheses, both unicellular and multicellular organisms are allowed to react. Plants are referred to as nature's chemical factories due to their low cost and low maintenance requirements. Since even trace amounts of these heavy metals are harmful, plants have shown great promise in detoxifying and accumulating them, which may help combat environmental contamination.[3] Plant extract-assisted metal nanoparticle synthesis has advantages over other biological synthesis methods, such as microorganisms, because it is more persistent, substantially quicker, and highly mono-dispersive.[4] The toxicity of certain bacteria, the isolation procedure of microorganisms, and the lengthy incubation process, making them unsuitable for many researchers. Plant extracts are therefore a remarkable source of metal and metal oxide nanoparticles. Additionally, the reaction kinetics of plant-assisted nanoparticle synthesis are significantly faster than those of other biosynthetic methods that are comparable to the production of chemical nanoparticles. Plant parts like fruit, leaves, stems, and roots have been widely used for the environmentally friendly method of producing nanoparticles because they produce high-quality phytochemicals.[5] Transforming metallic ions into nanoparticles and serving as reducing and stabilising agents. The electrons generated by the plant extract are responsible for the reduction of copper salts. Copper oxide nanoparticles are created when reduction takes place between phytochemicals and copper ions. Table 2 lists the primary advantages of using various plants for the biomediated synthesis of copper oxide nanoparticles.[6]

Sl. No.	Plant extract	Precursor	Part used	Size of NPs	Shape	Characterization techniques used	Ref.	
1.	*Carcica papaya L*	$CuCl_2$,$2H_2O$	Leaves	20nm avg.	Spherical & Crystaline	UV-Vis , XRD, FTIR, SEM , TEM	[7]	
2.	*Citrus medica L.*	$CuSO_4.H_2O$	Fruit	20nm avg.	Crystaline	UV-Vis, NTA & XRD	[7]	
3.	*Euphorbia esula L*	$CuSO_4.H_2O$	Leaves	20-110nm	Crystaline	UV-Vis , XRD, FTIR,	[8]	
4.	*Nerium oleander*	$CuSO_4.H_2O$	Leaves	9nm avg.	Crystaline	XRD, FTIR, SEM	[9]	
5.	*Ocimum tenuiflorum*	$CuSO_4.H_2O$	Leaves	72nm	Hexagonal crystaline	UV-Vis , XRD, FTIR, SEM,EADX	[9]	
6.	*Mangolia Kobus DC*	$CuSO_4.H_2O$	Leaves	37-110nm	Spherical	UV-Vis, ICP, EDS, XPS, HR-TEM	[10]	
7.	*Ginko biloba L.*	$CuCl_2$,$2H_2O$	Leaves	15-20nm	Spherical	UV-Vis, ICP, EDS, XPS	[11], [12]	
8.	*Artsbotrys odoratissimus*	$CuSO_4.H_2O$	Leaves	135nm	Spherical	PSA	[12], [13]	
9.	*Cinamon sp.*	$CuSO_4$	Leaves	18.17-91.28nm	Spherical	UV-Vis, DLS	[14]	
10.	*Inula helenium*	$CuSO_4$	Leaves	32.41nm	Spherical	UV-Vis, DLS	[13]	
11.	*Urtica sp.*	$CuSO_4$	Leaves	6.5 nm	Spherical	UV-Vis, DLS	[13]	
12.	*Glycyrrhiza glabra L*	$CuSO_4$	Leaves	28.21nm	Spherical	UV-Vis, DLS	[13]	
13.	*Eucaliptus sp.*	$CuSO_4$	Leaves	38.62nm	Spherical	UV-Vis, DLS	[13]	
14.	*Glycine max L.*	$CuSO_4$	Seeds	20nm	Spherical	UV-Vis, DLS	[15]	
15.	*Zingiber officinale roscoe*	$CuSO_4$	Rhizome	20-30nm	Spherical	UV-Vis , XRD, FTIR,	[14], [15]	

Table1.2 Biosynthesis of Cu/CuO nanoparticles by different plants.

Metal nanoparticles are a common nanotechnological object nowadays. The so-called "quantum size effects" are responsible for the fundamental changes in matter that occur when it reaches the nanoscale. These changes include changes in magnetic and optical properties, specific thermal capacity, melting point, and catalytic and surface activity. The state density in the conductance and valency zones changes significantly when a body shrinks in size from a macroscopic body to a few hundred or a thousand atoms. This alters the properties determined by the behaviour of electrons, mainly the electric and magnetic ones. At the macro level, the "continuous" state density is replaced by discrete levels, whose separations are based on particle size.[16]

1.1.1. Bottom-Up Green Methods: Nanoparticles are synthesized from atoms or molecules that self-assemble into nanoscale structures.

Biological techniques: using bacteria, fungus, algae, or plant extracts.

Reduction techniques: green reducing agents derived from natural sources, such as flavonoids, phenols, and ascorbic acid, are used to reduce copper salts, such as $CuSO_4$.

The mechanism:

- Copper atoms (Cu^0) are produced by reducing copper ions (Cu^{2+}).
- These atoms develop into nanoparticles by nucleation.
- The extract's biomolecules stabilise and cap the nanoparticles.

For instance: Using reducing and stabilising substances like as aloe vera, green tea, and neem leaf extract.

Benefits:

- Environmentally friendly
- Conditions for mild reactions
- Biocompatible
- Scalable

1.1.2. Top-Down Green Methods: Using mechanical or physical methods, bulk copper is reduced to nanoparticle size.

Biological Techniques:

- Though less frequent in green synthesis, green top-down methods are still feasible.
- Environmentally friendly mechanical milling.
- Green solvents, such as water or plant extract, are used for laser ablation.
- Green medium electrochemical etching.

The mechanism:

- Bulk copper is exposed to energy (thermal, mechanical, etc.).
- It breaks down into tiny particles.
- Aggregation can be avoided by using stabilising agents.

For instance:

- Copper is ball milled while biodegradable capping agents are present.
- A copper target immersed in a plant extract is laser-ablated.

Benefits:

- Accurate control of size (using top-tier equipment).
- Using bulk copper eliminates the requirement for copper salts.

Drawbacks:

- High energy usage
- Less influence over form
- Frequently less "green" than bottom-up approaches

1.2. THE VALUE OF COPPER FOR PLANTS

The microelement copper (Cu), which is present in block D and period 4 of the periodic table, is necessary for plant development. Under physiological conditions, it exists as Cu+ and Cu 2+. For a plant to develop normally, the concentration must be between 10-14 and 10-16 M; below this, a deficit takes place[17]. However, higher than optimal concentrations demonstrated toxicity in the absorption of nutrients. It takes part in oxidative stress response, hormone signalling, mitochondrial respiration, cell wall metabolism, photosynthetic electron transport, and a number of enzymatic reactions that are carried out by enzymes such as superoxide dismutase, plastocyanin, laccase, polyphenol oxidase, and amino oxidase. It also plays a structural role in the regulation of proteins. At the cellular level, it is essential for oxidative phosphorylation, iron mobilisation, and signal trafficking. In addition to curled leaves, downward-pointing petioles, and mild chlorosis, plants with a Cu deficit also permanent loss of turgor in immature leaves. Rosette growth happens when there is a persistent Cu shortage. shows the effects of copper on plants[18]. Oxidative stress, growth inhibition, toxicity, and disruption of photosynthesis and photorespiration are all brought on by elevated copper levels. Since Cu deficiency in plants results in yield losses with little to no display of the usual symptoms, diagnosis is essential. The dosages used ten to thirty years ago would be running low, and increased use of nitrogenous fertilisers would exacerbate the Cu shortage, potentially increasing its frequency in the future.[18], [19]

1.3. The process of COPPER NANOPARTICLE biosynthesis

Modern nanotechnology must create a dependable, non-toxic, clean, and environmentally

acceptable substitute for chemical synthesis. Biological synthesis is one technique that uses capping and reducing chemicals[19], [20]. such as plants, bacteria, fungus, actinomycetes, and yeast. Compared to chemical synthesis, there is a dearth of information on the biosynthesis of Cu Nps and other metal Nps[20], [21], [22].

1.3.1. Phytosynthesis-produced copper nanoparticles

Copper nanoparticles have been produced by phytosynthesis using a variety of plants and copper salts, such as copper acetate, copper sulphate, and copper nitrate. Calotropis procera latex and Aloe vera flower broth were utilised to produce copper nanoparticles with sizes of 15 and 40 nm, starting with copper acetate as the substrate[22]. When *Centella Asiatica* leaf extract was mixed with copper acetate, copper nanoparticles ranging in size from 2 to 5 nm were produced. Copper nanoparticles with a diameter of 5 to 93 nm were created phytochemically using copper sulphate and leaf extract from *Syzygium aromaticum, Tabernaemontana* divaricate, Vitis vinifera, and brown algae. *Bifurcaria bifurcata* extract and citrus medica fruit juice have been created.[23]Copper sulphate was added to Cassia alata flower extract to create copper oxide nanoparticles with a diameter of 110–280 nm. Using tea leaf extract, coffee powder, copper nitrate, Copper oxide nanoparticles with a size range of 50 to 100 nm were synthesised from Andean blackberry fruit and leaves.[24] Copper nanoparticles with sizes ranging from 5 to 10 nm were made using extract from *Gloriosa superba* and cupric nitrate. A thorough description of the various salts and plant materials used in copper nanoparticle phytosynthesis.

1.3.2. Biosynthesis of copper nanoparticles using microorganisms

Because of their varied biochemistry, ease of use, and potential for producing nanoparticles, microorganisms along with rapid cultivation under scientific conditions. Microorganisms can be used in a variety of ways to biosynthesise nanoparticles. A bacterium from the genus *Serratia*, which was isolated from the bug *Stibara* species, was the first known microbe to synthesise copper nanoparticles with a diameter of 10–30 nm using 5 mM copper sulphate. Pseudomonas *stutzeri* produced spherical copper nanoparticles with a diameter of 8 to 15 nm, whereas *Morganella morganii* and *Escherichia coli* produced quasi-spherical copper nanoparticles with a diameter of 10 to 40 nm.[25]. Copper sulphate and extracellular metabolites from Penicillium *aureogriseum, P. citrinum*, and *P. waksmanii* were used to create copper nanoparticles with a diameter of 79–295 nm. Dead biomass from *Hypocrea lixii* and *Trichoderma koningiopsis*, respectively, was used to create copper nanoparticles with sizes of 24.5 and 87.5 nm[26]. Salmonella typhimurium and Pseudomonas fluorescens MTCC103 culture supernatant were used to create copper nanoparticles, which had an average size of 49 nm. A fungus called *Stereum hirsutum* and copper salts were used to create copper/copper oxide nanoparticles, which ranged in size from 5 to 20 nm[27]. A summary of the requirements for biosynthesis, microorganisms, and salt concentrations is provided in. The analysis above made it abundantly evident that copper nanoparticles of various sizes and shapes have been created from a variety of plant materials and microorganisms, including bacteria and fungus.[28]Although the nanoparticles can be made using extracts from other plants, microbial synthesis ought to be given priority because commercial biosynthesis uses. Because they need a lot of raw materials, plant extracts are not a practical solution. Since microorganisms can be grown and multiplied in a lab environment and are controlled and managed for optimal nanoparticle output, they are better suited for the commercial production of copper nanoparticles.

Plant Material	Salt & Conc.	Syntheis Conditions	Size	Ref.
Calotropis procera L.	30 mM $Cu(CH_3COO)2$	Added latex to $Cu(CH_3COO)2$ with shaking	15nm	[29]
Syzygium aromatricam	0.001 M $CuSO_4$	Added extract to $CuSO_4$ and incubate for 1 hr.	5-40 nm	[27], [30]
Tabernaemontana divaricate	$CuSO_4$	Added extract to $CuSO_4$ with con. stirring	46 nm	[27]
Vitris vinifera	1 mM $CuSO_4$	Added extract to $CuSO_4$ incubate in dark room	-	[31]
Cassia alta	1.0 M $CuSO_4$	Added $CuSO_4$ to extract under sterring and incubate at 80°c	110- 280 nm	[32]

Table 1.3.1 Copper nanoparticle phytosynthesis: over the list of some plant materials that are used to make copper nanoparticles of various sizes from various salts.

1.4. The impact of copper nanoparticles on crops

The effects of nanoparticles on plants depend on a variety of factors. Criteria that include the plant species under investigation as well as the size, composition, concentration, and physical and chemical properties of the nanoparticles Although DNA damage in living organisms is mediated by metal and metal oxide-based nanomaterials, little is understood about the molecular mechanisms underlying this damage [33]. Copper nanoparticles at different concentrations (200–1000 mg/l) had a negative effect on the development of plant seedlings of Phaseolus radiates and Triticum aestivum in a controlled laboratory environment. Copper oxide nanoparticles inhibited the germination of seeds from Raphanus sativus, Lolium perenne, and Lolium rigidum in glycine. max and Cicer arietinum up to 2000 ppm of copper oxide nanoparticles smaller than 50 nm, but root development was inhibited over 500 ppm. At 50–500 mg/l, copper oxide nanoparticles reduced Glycine max growth by half. *Murashige & Skoog* medium. Copper oxide nanoparticles dramatically reduced the biomass and total chlorophyll content of *Arabidopsis thaliana* plants at concentrations greater than 2 mg/l. However, anthocyanin, lipid peroxidation, and amino acid proline content increased at concentrations greater than 5 mg/l[30]. Copper nanoparticles increased the mitotic index of Allium cepa cells that were actively proliferating up to 20 lg/ml, and as the concentration increased, it progressively decreased. Copper oxide nanoparticles dramatically improved germination, shoot, and root length when applied to damaged (artificially aged) maize hybrid cv. Hema seeds. Copper nanoparticles ranging from 0.2 to 1.0 ppm mixed in Murashige and Skoog medium greatly improved the agronomic parameters of wheat; however, concentrations above 2 ppm had adverse effects[34]. On the other hand, adding copper nanoparticles to the soil in pots at concentrations between 10 and 30 ppm significantly increased wheat growth and yield. A dangerous reaction happened when Oryza sativa and chickpea seedlings were exposed to concentrations of copper oxide nanoparticles exceeding 50 mg/l.[33] Copper oxide nanoparticles significantly slowed the growth of both conventional and transgenic cotton at concentrations greater than 10 mg/l. However, nanoparticles of copper oxide raised the

exogenous gene that encodes the Bt toxin protein in leaves and roots at low concentrations of 10 mg/l, giving Bt cotton insect resistance. After being exposed to copper oxide nanoparticles, the aquatic macrophyte Lemna gibba plants' ability to photosynthesise was reduced. With very few exceptions, copper nanoparticles have little effect on plant germination and growth, according to the literature review on the subject. However, studies on the effects of copper nanoparticles are becoming increasingly important in order to explore the use of nanoparticles for disease management without harming plants.

1.5. **Utilising copper nanoparticles to address diseases**

Numerous bacterial and fungal infections damage agricultural crops, reducing their production. Interest in evaluating the potential of nanoparticles for disease management in agriculture has grown as a result of the antibacterial activity demonstrated by copper nanoparticles against a wide variety of pathogenic microorganisms, including fungus and bacteria. According to studies on the in vitro antifungal efficacy of copper nanoparticles, Curvularia lunata MTCC 2030 had the highest antifungal activity, followed by Alternaria alternata MTCC6572, Fusarium oxysporum MTCC1755, and Phoma destructive DBT66[26]. Citrus medica-derived copper nanoparticles also showed inhibitory efficacy against Fusarium culmorum, F. oxysporum, and F. graminearum, three plant harmful fungus. With a 25 mm zone of inhibition, copper nanoparticles had the most antifungal efficacy against Fusarium equiseti, followed by F. oxysporum and F. culmorum.[33] After nine days of incubation, copper nanoparticles with a size of 20–50 nm and a concentration of 450 ppm were able to 93.98% suppress the development of Fusarium sp. Fungicides based on copper have been used to treat and prevent diseases in a variety of plant species. Three distinct copper-based nanoparticles with comparable sizes (11-14 nm) and shapes (Cu/Cu2O) were used in field tests conducted under protected cultivation against Phytophthora infestans on Lycopersicon esculentum. The results demonstrated that the copper-based nanoparticles outperformed the four registered copper-based agrochemicals[32], [35]. In addition to its encouraging effectiveness, it was discovered that plants were not permanently harmed or adversely affected by copper-based nanoparticles. The investigation of copper nanoparticles for disease control is just the start; much more work has to be done to sustainably protect plants from a range of illnesses.[32], [36], [37]

Chapter-2

Review Of Literature

2.1. The purpose and vital features of nanoparticles:

In a comprehensive review **Ahmed Abdelhalim Yameny** states that Ultrafine particles with sizes between one and one hundred nanometres are called nanoparticles. These materials' high surface area-to-volume ratio and quantum effects give them special physical, chemical, and biological characteristics. Nanoparticles with enzyme-like catalytic activities are referred to as "nanozymes" and fall into two primary categories: those that rely on the surface catalytic properties of inorganic nanomaterials and those that are immobilised catalysts on nanomaterials. The increasing specialisation and sophistication of nanotechnology are reflected in the changing nomenclature. Physical characteristics, morphology, and composition can all be used to categorise nanoparticles. Their distinct behaviour creates new opportunities for scientific research and technological advancement.[38]

2.2. Approaches for Nanoparticle Preparation: Nanoparticle synthesis uses various methodologies for size control, morphology, composition, and scalability, categorized into physical, chemical, and biological approaches. Researchers continuously develop new methods for improved precision and efficiency.

2.2.1. Physical Methods: When producing nanoparticles, physical methods like mechanical milling, laser ablation, and vapour deposition are employed to either break down bulk materials into nanoscale components or condense vaporised materials into nanoparticles. Through forward transfer generated by optical vortices, two-dimensional microprinting of ultrafine metallic dots is achieved.[39]

2.2.2. Chemical synthesis: methods, such as reduction, precipitation, solgel, and polymer-based nanostructures like polymersomes, use chemical reactions to create nanoparticles. These techniques give researchers exact control over particle properties, enabling them to customise nanoparticles for particular uses.[40]

2.3. Green synthesis:

provides an environmentally friendly alternative to traditional methods by using biological organisms like bacteria, fungi, or plants to create nanoparticles. This method is popular due to its affordability, simplicity of use, and low environmental impact. Plant extracts rich in phytochemicals, such as flavonoids, terpenoids, and polyphenolic compounds, act as reducing agents by converting metal ions into atoms that aggregate to form nanoparticles. Litchi chinensis extract has worked well for this process. The basic mechanics of green synthesis are now being investigated.

The study conducted a qualitative phytochemical analysis of methanol extracts from *P. argyreae, P. confusa, P. vittata, P. biaurita, and P. multiaurita,* followed standard procedures, and quantified the phytochemicals present in these extracts.

In this study, a qualitative phytochemical analysis was performed on methanol extracts from five ferns: *P. argyreae, P. confusa, P. vittata, P. biaurita, and P. multiaurita.* The extracts

produced positive results in ten phytochemical tests. The extracts contained **amino acids, reducing sugars, flavonoids, saponins, tannins, phenolic compounds, triterpenoids, steroids, sugars, alkaloids, and anthroquinones.** These compounds are vital secondary metabolites that give the plant its medicinal qualities. However, there was **no tannin** in the *P. multiaurita* extract. All extracts underwent additional analytical testing to measure the phytochemical components. [41]

The primary phytochemicals analysed in the study's examination of five fern extracts were **flavonoids, alkaloids, and phenolic compounds**. Preparations of Pteris species contained varying amounts of these compounds. **Flavonoids** were most abundant in all selected ferns, followed by **alkaloids** and **phenolic compounds**. The **tannins** and **saponins** were minuscule. Fern methanol extracts were filtered for phytochemical analysis. Prior research has been conducted on the phyto-constituents of Adiantum caudataum, Adiantum latifolium, Adiantum lunulatum, Christella dentate, and Christella parasitica. The amounts of **phyto-constituents** in these plants' crude extracts varied according to the plant extract solvents. According to the study, the selected Pteris species exhibited the highest concentrations of **alkaloids (16 mg), flavonoids (17 mg), and phenolics (13 mg).**[42]

2.4. Biomedical Uses of Copper Nanoparticles*

2.4.1. Antimicrobial Agents

Mechanism*: Degrades microbial membranes, generates reactive oxygen species (ROS), and binds to DNA and proteins.
Applications*: Filtration of water; * Hospital clothing (including antimicrobial masks and gowns); * Dressings and coatings for wounds.[43]

2.4.2. Cancer Therapies

Mitochondrial damage.
Applications*:Synergistic agents in chemotherapy and photothermal therapy * Nanocarriers for targeted drug delivery[44]

2.4.3. Uses for Antioxidants

Method*: Scavenges free radicals and prevents oxidative damage.

Applications : Possible use in the management of neurodegenerative diseases * Preservation of cells and tissues in inflammatory conditions[45]

2.4.4. Healing Injuries

Mechanism: Promotes angiogenesis, or the formation of new blood vessels, and collagen synthesis.
Applications: Applying nanoformulations in creams, gels, or sprays to diabetic ulcers and burns to promote faster healing [46]

2.4.5. Biosensors

Mechanism*: high electrical conductivity and catalytic activity.

Applications*: Identifying glucose, cholesterol, or cancer biomarkers * Point-of-care diagnostic instruments[47].

2.4.6. Antiviral Applications

*** Mechanism**: Stops replication by disrupting the proteins and viral envelope.

Applications:

Surface coatings and sprays: A range of surface coatings have been enhanced with CuNPs to give them antiviral properties.

Polymeric Spray Coatings: It was demonstrated that a polyurethane-based spray coating containing CuNPs could inactivate over 99% of SARS-CoV-2 particles within 30 minutes of contact.

With up to 100% inactivation rates, studies employing laser-generated CuNP coatings on surfaces have demonstrated significant virucidal efficacy against model herpesviruses and coronaviruses**.**

Additives for Personal Protective Equipment (PPE)

The addition of CuNPs to PPE has been studied as a way to enhance antiviral protection:

Face Masks: Adding CuNPs to mask filters has been shown to effectively inactivate SARS-CoV-2, providing an additional layer of defence against viral spread.[47], [48]

2.4.7. ⚠ *Challenges*

Because of their special qualities, copper nanoparticles (CuNPs) offer potential for use in biological applications. Among the difficulties they encounter include high concentration toxicity and unregulated Cu2+ ion release. These include organ-specific toxicity, oxidative stress and cell destruction, and toxicity that is depending on size and concentration. Environmental factors and synthesis techniques, such as oxidation and aggregation in aquatic settings, affect the stability and biodegradation of CuNPs. The synthesis techniques can also affect the physicochemical characteristics of CuNPs. For CuNP applications to be consistently reproducible, standardised synthesis techniques are required. Due to variations in biological materials, green synthesis techniques and property variability may be introduced. For the safe and efficient integration of CuNPs in biological applications, these issues must be resolved by thorough study and standardised procedures**.**[49], [50]

2.5. Environmental Applications

2.5.1.Water purification and wastewater treatment

2.5.1.1. Biosynthetic Production of CuNPs for Wastewater Treatment: In a study that synthesised CuNPs using a bioflocculant derived from Proteus mirabilis, significant removal rates for pollutants such as BOD (90%), COD (87%), phosphates (88%), and nitrates (80%) from coal mine effluent were demonstrated, along with excellent flocculation efficiency (97%). [49]

2.5.1.2 Water Purification at the Point of Use with CuNP-Integrated Paper Filters: Researchers developed paper filters studded with CuNPs to effectively inactivate Escherichia coli germs in water, yielding an 8.8 log decrease. Copper concentrations in the treated water remained below recommended levels for drinking water.[51]

2.5.1.3. Removing Dye with Green-Synthesized CuNPs : CuNPs derived from Trapa natans L. shells were able to effectively remove colours like methyl orange and brilliant green from aqueous solutions when added to polyamide-12 composites, indicating that they could be used to clean industrial effluent**.**[52]

2.5.2. 🧪 Organic Pollutant Removal

2.5.2.1 Removal of Polycyclic Aromatic Hydrocarbons (PAHs): By removing PAHs from bitumen-contaminated water with high adsorption efficiencies (up to ~84%), CuNPs made from extracts of Echinochloa pyramidalis demonstrated promise in the remediation of organic pollutants**.** [53]

2.5.2.2. Using Nano-Pesticides to Fight Phytopathogens in Agriculture: Biogenically produced copper oxide nanoparticles showed significant antibacterial activity against a variety of plant infections, suggesting that they could be used as environmentally friendly nanopesticides in agriculture. [54]

2.5.2.3. Impact on Soil-Plant Systems: CuNPs can serve as micronutrients and protective agents in agriculture, but excessive use can jeopardise soil health and microbial populations. An in-depth examination highlights the importance of closely assessing their interactions within the soil-plant ecosystem.[55]

2.5.3. Environmental Security and destiny

2.5.3.1. The Impact of Environmental Factors on CuNP Behaviour: The behaviour of CuNPs in the environment depends on a number of factors, including particle size, production

methods, and the presence of organic matter. These factors affect their stability, aggregation, and potential toxicity in soil and aquatic environments.[56]

2.6. Copper/copper-oxide nanoparticles (Cu/CuO NPs) are formed by pteris: The optical, antibacterial, and catalytic activities of copper nanoparticles (Cu NPs) are greatly influenced by their physicochemical characteristics, which include their shape, particle size, surface area, crystallinity, and dispersion. The following characteristics are commonly seen in CuO NPs that are synthesised utilising Pteris extracts:

- Their particle sizes typically range from 20 to 100 nanometres, though finer control during synthesis can produce smaller sizes. They frequently have hexagonal wurtzite crystal structures and display spherical, rod-like, or irregular morphologies, depending on the phytochemical composition of the extract and synthesis parameters.
- Depending on the content of biomolecules (such as proteins, flavonoids, and polyphenols) in the Pteris extract, which serve as both stabilising and reducing agents, the nanoparticles may exhibit excellent dispersion or mild aggregation.
- The abundance of biomolecules (such as proteins, flavonoids, and polyphenols) in Pteris extract, which function as both stabilising and reducing agents, may cause the nanoparticles to exhibit excellent dispersion or minor aggregation.[57]

2.6.1. 🔬 CuO Nanoparticles Made with Pteris Extracts: Physical-chemical Characteristics

2.6.1.1. Particle Size and Shape:

- CuO NPs typically range in size from 20 to 100 nm.
- Depending on the extract content and synthesis conditions, the morphologies can be round, rod-like, or irregular.
- For instance, CuO NPs synthesised from Pteris vittata extract displayed spherical and rod-like shapes with diameters of about 50 nm.

2.6.1.2. Crystallinity and Structure:

- X-ray diffraction (XRD) analysis often reveals a hexagonal wurtzite crystal structure.
- Studies have confirmed the monoclinic phase of CuO with characteristic peaks at 2θ values of 32.5°, 35.6°, 38.7°, 48.9°, 58.3°, 66.4°, 68.1°, 72.5°, and 75.1°, which correspond to the (−111), (002), (111), (−202), (202), (−311), (113), (311), and (400) planes.

2.6.1.3. Chemistry and Surface Dispersion:

- Proteins, flavonoids, and polyphenols are among the biomolecules found in the Pteris extract that act as capping and reducing agents.
- These compounds have an impact on the stability and dispersion of the nanoparticles, leading to either widely distributed or minimally aggregated formations. [58]
- In Fourier-transform infrared (FT-IR) spectroscopic studies, Cu–O bond peaks are commonly observed, indicating that these biomolecules contribute to the formation of nanoparticles.[58]

2.7. Mechanisms of Copper or Copper Oxide Nanoparticles' Antibacterial Action Pteris-Synthesized:

Copper or copper oxide nanoparticles (Cu/CuO NPs), which have shown great promise as antimicrobial agents, have been produced using plant extracts from Pteris. These eco-friendly nanoparticles are effective against a range of harmful microbes, including: Gram-positive bacteria, including *Staphylococcus aureus* and *Bacillus subtilis* Gram-negavites, including *Pseudomonas aeruginosa* and *E. coli*

- Fungal infections, including Candida albicans.
- Some drug resistance even develops in bacterial strains.

The strong antibacterial action of these Cu/CuO NPs is believed to work through several mechanisms[59]

2.7.1. The Mechanisms of Antibacterial Activity

2.7.1.1. **ROS (Reactive Oxygen Species) Production:** By generating ROS, such as hydroxyl radicals and superoxide anions, CuO NPs can put bacteria under oxidative stress. When these reactive species damage cellular components, cell death occurs.

2.7.1.2. Cell membrane integrity: It can be compromised by CuO NPs' interaction with bacterial cell walls, which can increase membrane permeability and allow essential intracellular materials to leak out, ultimately resulting in cell lysis.

2.7.1.3. DNA and Protein Interaction: CuO NPs can bind to the DNA and proteins of bacteria, blocking enzymatic and replication processes, which hinders the bacteria's ability to grow and survive.

2.7.1.4. Cu2+ Ion Release: When CuO NPs dissolve, Cu2+ ions are released. By interacting with biological elements, these ions can increase their ability to fight bacteria in.

2.8. The Role of Pteris Extracts in CuO NP Synthesis

When producing CuO NPs in an environmentally friendly manner, bioactive materials like proteins, flavonoids, and polyphenols that are present in pteris plant extracts act as stabilising and reducing agents. These biomolecules influence the size, shape, and surface properties of the nanoparticles, which in turn influences how well they combat bacteria.[59], [60]

2.8.1. Research Studies That Are Relevant

Understanding the mechanism of the antibacterial activity of copper oxide nanoparticles: This study investigates the mechanisms by which CuO NPs combat bacteria, highlighting the functions of membrane rupture and ROS generation.

2.8.2. Copper Oxide Nanoparticles Green Synthesis, Characterisation, and Antimicrobial Activity:

This study emphasises how size and shape of nanoparticles affect antibacterial activity, demonstrating that larger surface areas make nanorods more effective.[61]

2.8.3. Green synthesised copper oxide nanoparticles' antibacterial efficacy against multidrug-resistant bacteria:

This study examines how well plant-mediated CuO NPs work against resistant bacterial strains, attributing its success to processes including membrane rupture and ROS generation.[62]

2.9. The Impact of copper nano Synthesis using pteris extract Parameters:

A sustainable and ecologically friendly approach to nanotechnology is the synthesis of copper nanoparticles (CuNPs) using plant extracts, such as those from the Pteris genus. Although there are few specialised studies on Pteris extracts, research on related pteridophytes, like Marsilea quadrifolia, provides valuable insights into the synthesis factors and their effects on CuNP properties.

2.10. Important Elements Influencing the Synthesis of CuNPs Using Pteris Extracts

2.10.1. Phytochemical composition

The presence of bioactive compounds like flavonoids and phenolics in plant extracts is crucial for the reduction of copper ions and the stabilisation of the resulting nanoparticles. By serving as capping and reducing agents, these compounds have an impact on the size and stability of CuNPs.

2.10.2. pH levels

The pH of the reaction mixture has a significant impact on the size and shape of the CuNPs that are produced. Smaller, spherical nanoparticles are frequently created because Cu2+ ion reduction rates are higher at higher pH values. Conversely, lower pH levels may produce larger, triangular or rod-shaped nanoparticles.[63]

2.10.3. The temperature

The synthesis of CuNPs is accelerated by higher temperatures because they speed up the reduction process. However, the stability and homogeneity of the nanoparticles may be jeopardised by aggregation caused by extremely high temperatures. [64]

2.10.4. Concentration Ratios

The ratio of copper salt solution to plant extract controls the nucleation and growth of nanoparticles. Higher concentrations of plant extract can cause rapid reduction, which results in smaller nanoparticles. An ideal equilibrium is needed to ensure consistency and prevent agglomeration. [64]

2.10.5. Reaction Time

The length of the reaction affects the size and crystallinity of CuNPs. Longer response times may lead to an increase in larger particles, while shorter ones may cause a partial reduction.[63]

2.11. Comparative Efficiency of Cu NPs:

Research on copper oxide (CuO) nanoparticles made with Pteris extracts is limited, but studies on CuO nanoparticles made environmentally with other plant extracts provide valuable insights into their applications and relative efficacy. Here are some pertinent studies:

2.11.1. The application of antimicrobial coatings to surgical tools and bandages: CuO nanoparticles prepared with Tabernaemontana divaricata leaf extract demonstrated strong antibacterial activity against the common urinary tract infection Escherichia coli. The study illustrated the potential of these biogenic nanoparticles to develop antibacterial coatings for therapeutic applications. [27]

2.11.2. Second, water purification agent disinfection systems : CuO nanoparticles were produced using an algal extract from Coelastrella terrestris. They effectively degraded wastewater's colours and showed enhanced photocatalytic activity. They can also be utilised in water filtration systems due to their antimicrobial properties.[65]

2.11.3. Antimicrobials applied topically to treat skin infections and inflammation: According to a study that produced CuO nanoparticles using Artemisia annua aqueous extract,

they have the potential to be topical antibacterial agents. They also showed antioxidant properties and burn wound healing activities.[66]

2.11.4. Slow-Release Platforms for Delivery of Drugs : Research on phyto-mediated CuO nanoparticles focusses on how they functionalise with organic compounds to facilitate drug conjugation. They can be used to develop slow-release drug delivery systems that increase the efficacy of antibiotics because of this feature. [67]

2.11.5. Sustainable Agriculture: Nano-Pesticides or Biofertilizers: Despite having no direct relationship to Pteris extracts, research has shown that plant-based CuO nanoparticles may be effective antimicrobial agents in agriculture, suggesting their potential as biofertilizers or nano-pesticides.[67]

Chapter-3

MATERIALS & METHODS:

3.1 Objectives

1. **Forming Cu/CuO NPs using *pteris* extract.**
2. **Isolation & Purification of formed nano-particles.**
3. **Characterization of Isolated nano-particles using XRD analysis, DLS, FTIR & UV-Vis Spectrophotometer.**
4. **Examine It's antimicrobial activity with the help of Broth Dilution Assay & Agar Diffusion Assay.**

3.2. Materials:

Copper Sulphate Pentahydrate ($CuSo_4,5H_2O$), Plant extract, Distilled water, NaOH, HCL, Nutrient broth (NB), Luria-Bertani broth (LB), Iodonitrotetrazolium chloride (INT dye), DMSO, KBr.

3.3. Sample Collection:

We Collected plant material from the South 24 Parganas district, especially around Dinabandhu Andrews College in Garia, Kolkata, West Bengal, India (Latitude: 22.4491° N, Longitude: 88.3915° E). Boral, Baishnabghata, Narendrapur, and New Garia are some of the communities nearby that are known for having both urban and semi-natural green spaces. These areas are home to many different types of plants and are great places for ferns and other plants to grow.

When the weather was good, the collection was done by hand during the day. We carefully picked mature and healthy plant specimens to make sure they had the most phytochemicals and biological activity possible. The plant material was put into sterile, labelled polythene bags right after it was picked and brought to the lab in normal conditions to keep it fresh and stop it from going bad. Every step of the collecting and transporting process was taken to make sure that nothing got contaminated.

Fig3.3. Pteris plant

3.4. Sample Identification

The plant specimen was identified taxonomically with the help of knowledgeable academic members from the Department of Botany at Dinabandhu Andrews College in Garia, Kolkata, West Bengal, India (Pin 700084). It was necessary to carefully look at the physical features and compare them to common botanical references and herbarium specimens as part of the identification process. To make sure that the identified specimen was real and could be used again, it was given a special voucher number and stored in the Herbarium at Dinabandhu Andrews College's Botany Department. This voucher specimen will be used to help with more research and verification.

3.5. Preparation of Salt Solution:

In order to produce 0.1M $CuSO_4$ salt solution we've to weigh proper conc. of Copper-Sulphate Pentahydrate salt. As we know that the molecular weight of Copper-Sulphate Pentahydrate was 159.609 g/mol so, the accurate weight of $CuSO_4$ is 15.96g and this conc. of need to be mixed into 1000ml of Distilled water ($D.H_2O$). At first the salt need to be poured into 600ml $D.H_2O$ and stir it maintaining aseptic conditions , when the salt is dissolved into that 600ml $D.H_2O$ then add rest 400ml $D.H_2O$ and our 1ltr (600+400=1000ml) 0.1M $CuSO_4$ salt solution is ready to use.[68], [69]

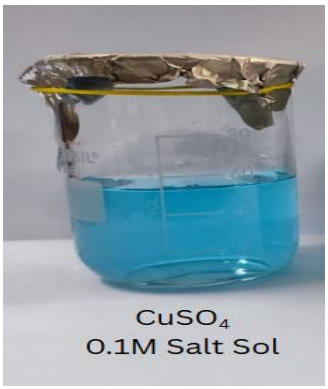

fig 3.5

3.6. Plant Extract Preparation:

Following collection, the Pteris leaves were carefully cleaned, first with tap water to remove any visible dirt and then with distilled water to remove any remaining surface impurity traces. The leaves were cleaned and then left to air-dry in the shade at room temperature (25 to 30 °C) for seven to ten days until they were crisp and completely dry. These dried leaves were then ground into a fine powder using a clean electric grinder and stored in an airtight container at room temperature until needed.

In a 500 mL Erlenmeyer flask, 20 grammes of the powdered leaves were combined with 200 millilitres of distilled water to create the aqueous extract. To extract the bioactive plant chemicals, this mixture was heated gradually for 30 minutes at 70 to 80 °C on a magnetic stirrer hot plate. After heating, the mixture was let cool naturally before being filtered using Whatman No. 1 filter paper to get rid of any remaining solids. The resultant transparent extract was put into amber-colored bottles and kept at 4 °C in a refrigerator. Later, this extract was employed in the environmentally friendly manufacture of Copper/ Copper oxide nanoparticles as a natural stabilising and reducing agent.[70], [71], [72]

Fig 3.6

3.7. Green Synthesis of Copper Nanoparticles (Cu-NPs)

1.First, the necessary amount of salt was dissolved in distilled water to create a new 0.1 M solution of copper sulphate ($CuSO_4 \cdot 5H_2O$) (mandatory).

2. To begin the synthesis, 90 mL of this copper sulphate solution was transferred into a sterile beaker. Using a magnetic stirrer, the mixture was constantly stirred as 10 mL of the Pteris leaf extract was gradually added. For one to two hours, the entire procedure was carried out at a

regulated temperature of around 60 to 80 °C. The solution's colour and purity gradually changed throughout this period, signifying the start of the production of nanoparticles.

3. The production of Cu nanoparticles was confirmed when a white precipitate began to develop at the beaker's bottom as the reaction went on. The liquid was allowed to cool to room temperature when the reaction was complete. After that, the nanoparticles were separated by centrifugation for around fifteen minutes at 10,000 rpm. The recovered precipitate was repeatedly cleaned with distilled water and ethanol to eliminate any remaining contaminants or plant residues in order to guarantee cleanliness.

4.To get fine Cu nanoparticle powder, the cleaned nanoparticles were then dried for 6–8 hours at 60 °C in a hot air oven. For additional testing and characterisation, the dried product was meticulously kept in airtight containers.[65]

The natural phytochemicals in the Pteris extract, which serve as stabilising and reducing agents, are the only source of energy used in this green synthesis method. It fits very nicely with ecologically friendly research procedures and provides a non-toxic and sustainable substitute for conventional chemical approaches.

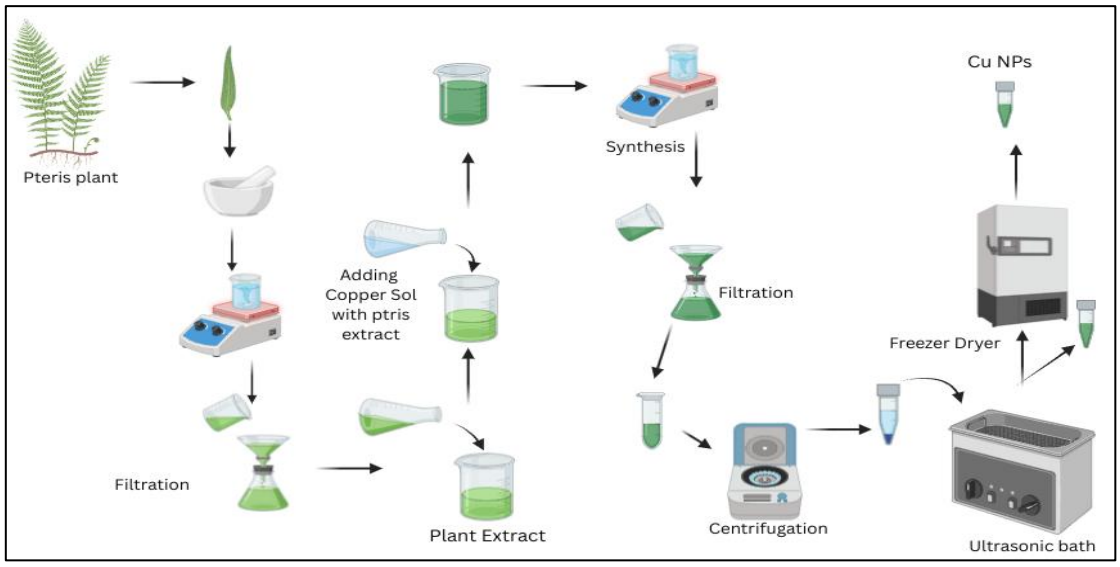

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Fig 3.7: Complete process of CuNPs

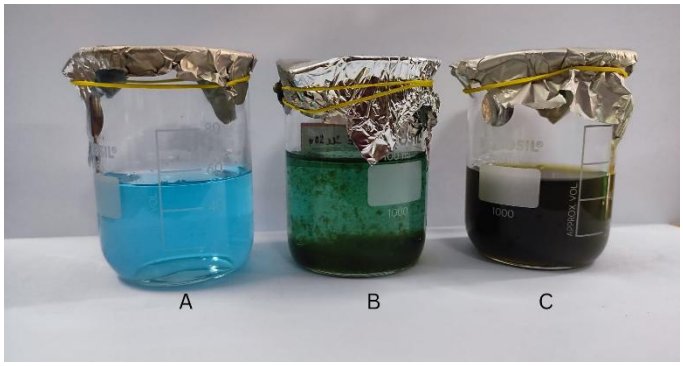

Fig 3.7.1: Specimen A- Copper Sulphate pentahydrate sol. (0.1M), Specimen B- Forming NPs of Copper Sulphate, Specimen C- Pteris extract

3.8. Characterization:

3.8.1. Characterization of Cu/CuO Nanoparticles: A number of common characterisation procedures were used in order to gain a better understanding of the characteristics of the green-synthesised zinc oxide nanoparticles. By identifying their characteristic optical absorption pattern, UV-visible spectroscopy was initially utilised to validate the creation of nanoparticles. FTIR analysis was used to determine which plant-based functional groups were responsible for stabilising and decreasing the nanoparticles. The CuO-NPs' crystal structure and purity were investigated by X-ray diffraction, and their size, shape, and general morphology were visualised by transmission electron microscopy. The average particle size and distribution in solution were ascertained by dynamic light scattering (DLS), which provided information on the particles' stability in a colloidal state. Together, these techniques provide a thorough comprehension of the produced nanoparticles, using methodologies akin to those reported in previous research.

1. **UV-Vis Spectroscopy of Green Synthesized Nanoparticles:** To verify the biogenesis and assess the optical characteristics of Copper/Copper oxide nanoparticles (Cu/CuO-NPs) made with Pteris leaf extract, UV-visible spectroscopy was employed as a conventional analytical method. Because of its efficiency in tracking the electrical transitions and surface plasmon resonance (SPR) behaviour typical of metal and metal oxide nanoparticles, this non-destructive technique is often used in nanoparticle research. The synthesised Cu/CuO-NP colloidal solution was examined without dilution in order to perform spectrum analysis. A calibrated double-beam UV-Vis spectrophotometer fitted with quartz cuvettes with a 1 cm path length was used to perform the measurements. To guarantee accuracy, baseline correction was carried out using distilled water prior to sample scanning. At room temperature, the absorption spectra were captured at wavelengths between 200 and 800 nm. Because it includes the area where Cu/CuO nanoparticles usually show a prominent absorbance peak corresponding to their band gap energy, the scanning range was selected based on existing literature. Finding this peak offers proof that nanoparticles are forming as well as information on the size and shape of the produced material. The approach follows

well recognised guidelines for characterising green nanoparticles, as documented in earlier research on plant-mediated Cu/CuO-NP production. When characterising biogenically synthesised nanoparticles, this technique guarantees scientific dependability and procedural consistency.[73]

2. **Dynamic Light Scattering analysis of Green Synthesized Nanoparticles:** The average hydrodynamic size and particle size distribution of the green-route copper/copper-oxide nanoparticles (Cu/CuO-NPs) made with Pteris leaf extract were evaluated using Dynamic Light Scattering (DLS). This method estimates the size of particles in a colloidal system by measuring variations in the intensity of scattered light and analysing the Brownian motion of suspended nanoparticles. To get rid of any dust or big clumps, the nanoparticle solution was first filtered via a 0.22 µm membrane filter. After filtering, the material was moved to a dust-free, sterile polystyrene cuvette for measurement. A particle size analyser with a 633 nm laser source and a fixed scattering angle of 90° was used to conduct the DLS analysis at room temperature. Two important parameters were revealed by the analysis: the polydispersity index (PDI), which shows the homogeneity of dispersion within the nanoparticle solution, and the Z-average diameter, which represents the mean hydrodynamic size of the particles. Good colloidal stability and particle size homogeneity, which are essential for later applications, are indicated by a narrow PDI. The DLS approach adhered to established protocols that are often used in research addressing the colloidal characterisation of metal oxide nanoparticles and green synthesis.[74]

3. **X-Ray Diffusion Analysis of Synthesized Nanoparticles:** The crystalline structure and phase identity of the Cu/CuO-NPs, which were made with Pteris leaf extract, were investigated using X-ray diffraction (XRD) analysis. XRD is very useful for studying nanoparticles because it is a tried-and-true technique for determining the arrangement of atoms in crystalline materials. After being finely ground to a uniform consistency for the analysis, the dried Cu/CuO nanoparticle powder was carefully transferred onto a sanitised XRD sample container. The data's clarity was improved and background noise was decreased by keeping the surface level and clean. The sample was scanned using an X-ray diffractometer that used copper K-alpha (Cu-Kα) radiation with a wavelength of 1.5406 Å. The machine was set to 40 kV and 30 mA, and the scan was performed over a 2θ range of 20° to 80° with a step size of 0.02°. These parameters allowed for the accurate identification of diffraction peaks. The visualised peaks were then compared to standard reference data from the Joint Committee on Powder Diffraction Standards (JCPDS) in order to ascertain the crystal structure of the nanoparticles. The presence of distinct peaks that matched the hexagonal wurtzite structure of Cu/CuO verified the product's effective synthesis and crystallinity.[75], [76]

4. **Fourier Transform Infrared Spectroscopic Analysis of Synthesized nanoparticles:** FTIR spectroscopy was used to determine which functional groups were in the Pteris leaf extract and to learn more about how they affected the stabilisation and reduction of Copper/Copper-oxide nanoparticles (Cu/CuO-NPs). Because of their distinctive absorption of infrared light, some chemical bonds and functional groups can be found using this approach. The study started with a 1:100 weight ratio of spectroscopic-grade potassium bromide (KBr) and a tiny amount of the synthesised and dried Cu/CuO

nanoparticle powder. A clean mortar and pestle were then used to grind this mixture finely so that it blended evenly. A hydraulic press operating at high pressure was then used to compress the resultant powder into a translucent pellet. An FTIR spectrometer was used to record the FTIR spectra at room temperature between 4000 and 400 cm^{-1}. To remove interference, the sample spectra were automatically subtracted from the background spectrum, which was measured using a pure KBr pellet. To identify the functional groups involved—especially those from the plant extract that may have served as stabilising and capping agents during the creation of the nanoparticles—the primary absorption peaks were closely. Examined.

Sl. No.	FTIR Spectral Regeion(cm^{-1})	Assigned Functional Groups	Ref.
1.	749-500	R-C-H	[77]
2.	941-803	C-H in plane deformation with atomic ring structure.	[78]
3.	1090-1049	-C-O-C-	[79]
4.	1260-1100	Amide III/ N-H bending with the C-N stretching vibration.	[79]
5.	1380-1330	CH_3 Deformation	[77]
6.	1490-1401	Symmetrical Stretching vibrations of -COO	[80]
7.	1580-1510	Amide II/ N-H Bending vibration.	[80]
8.	1700-1600	Amide I/ C=O Stretching vibration.	[81]
9.	2973-2541	CH_3 / C-H stretching Vibration	[82]
10.	3450-3250	N-H Stretching.	[81], [82]
11.	3600-3200	O-H Stretching of Alcohols/ Phenols	[82]

Table 3.8 Different Peaks of FTIR Test & Their indication

3.9. Antimicrobial Activity of Cu-NPs:

3.9.1. A thin layer of copper nanoparticles' antibacterial qualities: In this study, CuNPs were synthesised in a silica matrix to create a thin film that inactivated more than 99% of S. aureus and E. coli in just 1.5 hours. The primary mechanism was found to be oxidative stress brought on by singlet oxygen.[82], [83], [84]

3.9.2. Synthesis, Characteristics, and Antimicrobial Activity of Copper Oxide Nanoparticles:[83]

Researchers produced CuO nanoparticles with an average size of 23 nm and showed strong antimicrobial efficacy against a range of bacterial species, including E. coli, P. aeruginosa, and S. aureus.[84]

Two commonly used microbiological tests, the broth microdilution method and the agar dilution method, were used to evaluate the antibacterial properties of the biosynthesised Cu/CuO-nanoparticles. The effectiveness of the nanoparticles in preventing bacterial growth was ascertained by these assays. Using the agar dilution technique, bacterial colonies were dispersed over the surface of LB agar media containing varying quantities of Cu/CuO-nanoparticles. Any discernible suppression of bacterial growth following incubation was recorded as an indication of antibacterial action. LB broth mixed with p-iodonitrotetrazolium violet (INT) dye—which changes colour when metabolically active bacteria are present—was used in the broth dilution procedure for a more quantitative study.[85]The minimum inhibitory concentration (MIC) of the Cu/CuO-NPs—the lowest concentration that can stop observable bacterial growth—was found by looking at the dye's colour change. The antibacterial efficacy of the synthesised Cu/CuO-NPs was reliably measured by these approaches, which adhered to conventional protocols as outlined in previous studies on nanoparticle antimicrobial testing.[86]

3.9.3. Agar Diffusion Assay: Two model bacterial strains, Bacillus subtilis (Gram-positive) and Escherichia coli (Gram-negative), were used in an agar well diffusion method to evaluate the antibacterial capability of the biosynthesised Copper/Copper-oxide nanoparticles (Cu/CuO-NPs). The culture medium, nutrient agar, was made in accordance with the manufacturer's usual directions. Following a 15-minute autoclaving at 121°C to sterilise the medium, it was transferred onto sterile Petri dishes and left to harden in a hygienic setting. A turbidity of 0.5 McFarland was achieved by adjusting fresh overnight bacterial cultures in nutrient broth. To guarantee a constant microbial lawn, the cultures were dispersed uniformly across the surface of the solidified agar using sterile cotton swabs. Following inoculation, a sterile cork borer was used to gently create four wells in the agar, each measuring 10 mm in diameter. 50 µL of Cu/CuO-NP solutions at 25, 50, and 100 µg/mL, respectively, were added to three of the wells. To confirm that any antibacterial activity was caused by the nanoparticles alone, the remaining well served as a negative control and was filled with 50 µL of non-ionized distilled water. After that, the plates were incubated for 24 hours at 37 ± 1°C inverted. Following incubation, the plates were checked for clean areas surrounding the wells, which signify the suppression of

bacterial growth. Every test was carried out in triplicate to guarantee accuracy and dependability.

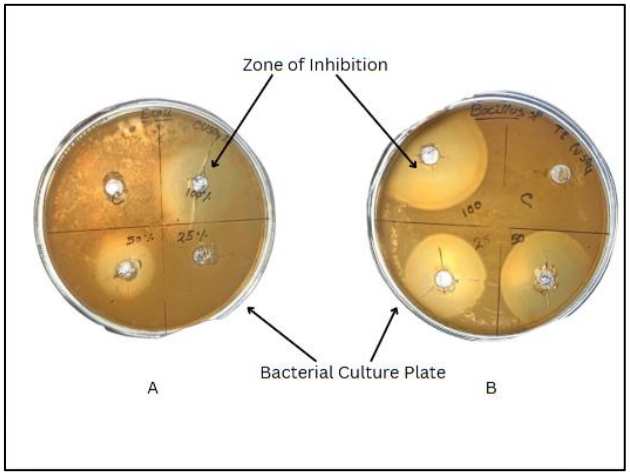

Fig 3.9.3: Bacterial culture plates treated by ZnNPs solution; A) *Escherichia coli* and B) *Bacillus subtilis*

3.9.4. Broth Dilution Assay: Using a broth microdilution technique, the antibacterial activity of the green-synthesised Copper/Copper-oxide nanoparticles (Cu/CuO-NPs) was further assessed against Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli. For this test, the culture medium was Luria-Bertani (LB) broth. A sterile 96-well microtiter plate was prepared with a 200 µL total volume in each well. 20 µL of fresh bacterial inoculum, standardised to meet 0.5 McFarland turbidity, was used for each treatment. The remaining 180 µL was made up of different ratios of Cu/CuO-NP solution and LB broth, depending on the concentration of nanoparticles being examined. The concentrations of nanodoses that were employed were 0.25, 0.5, 0.75, 1.0, and 1.25 mg/mL. For the control sets, 20 µL of bacterial inoculum was combined with 180 µL of LB broth; no nanoparticles were added. To maintain a consistent total volume of 200 µL for treated wells, the amount of LB broth was decreased in proportion to the concentration of the nanoparticle solution. The plates were incubated at 37 ± 1°C throughout the whole night. As a colorimetric measure of bacterial viability, 20 µL of an INT (iodonitrotetrazolium) dye solution was applied to each well after incubation. To ensure a proper dye response, the plates were then left in the dark for a further half hour. Visual observation of any colour shift was used to gauge the prevention of microbial growth. To guarantee uniformity and repeatability, each treatment was carried out in triplicate.[86]

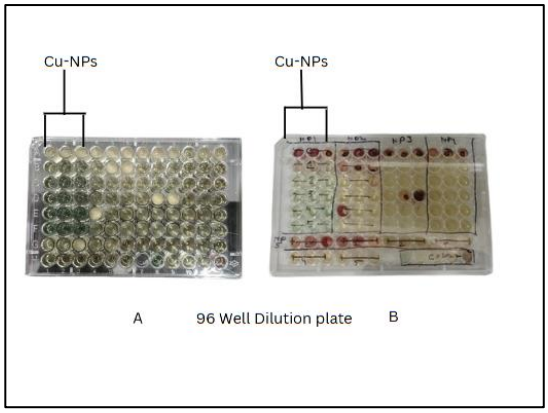

Fig3.9.4: bacterial culture treatment under 96- well dilution plate ; A) over night incubated plate, B) 30 mins dark incubated plate after apply INT dye.

Chapter-4

4.Results & Discussion

4.1.Copper-Nanoparticle's Green In-vitro synthesis :

Pteris extract was exposed to a 0.1 mM aqueous solution of copper sulphate (CuSO4) in order to produce copper nanoparticles in vitro in an environmentally safe manner. When the plant extract was added to the metal salt solution, a colour shift from dark green to dark-green occurred over time. This colour shift indicated the efficient biosynthesis of copper nanoparticles (Cu-NPs) and suggested the reduction of Cu2+ ions and the formation of stable nanoparticles. The visual change in the reaction mixture is caused by the stimulation of surface plasmon resonance (SPR), which typically takes place in conjunction with the formation of metal nanoparticles.

The colour shift was brought about by the formation of the nanoparticles rather than by any inherent properties of the component parts, as neither the *Pteris* extract alone (positive control) nor the copper-sulphate solution (negative control) displayed any colour change. [87] Similar biosynthetic techniques using different biological sources have been documented in the past. Outlined a comparable green synthesis of Cu-NPs using *Calotropis gigantea* leaf extract, where a noticeable colour shift signalled the creation of nanoparticles. Similar visual cues were observed when *Aspergillus flavus* was used to biosynthesise copper/copper-oxide nanoparticles. Another study demonstrated the synthesis of Cu/CuO-NPs using leaf extract from *Azadirachta indica*, demonstrating the efficacy of plant-based techniques for producing nanoparticles[88].

These findings are in line with our recent finding that plant extracts, which contain a wealth of phytochemicals such as flavonoids, terpenoids, and polyphenols, act as both stabilising and reducing agents during the nanoparticle production process. Because of its affordability, ease of use, and environmental friendliness, this technology is a good alternative to traditional chemical synthesis methods.

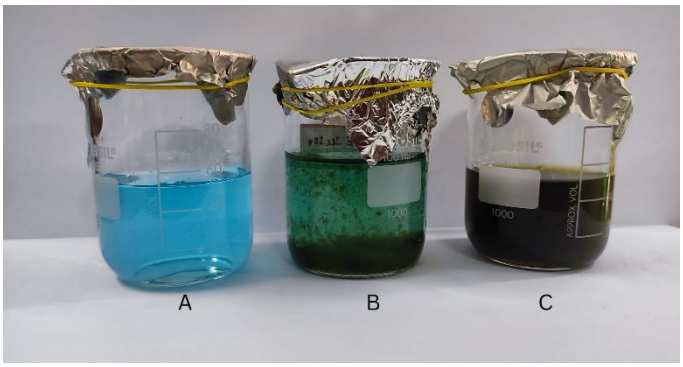

Fig 4.1: Visual change in colour of the reaction mixture compared with the control set; A) Copper-sulfate solution; B) reaction mixture; C) Pteris extract.

4.2.Characterization of the copper nano particles:

UV-Visible Spectrophotometric Analysis of Copper Nanoparticles Synthesized Using *Pteris* Extract:

1. **Surface Plasmon Resonance (SPR) Evidence:**
    - Absorbance at approximately 425 nm is commonly associated with the Surface Plasmon Resonance (SPR) of copper nanoparticles (Cu-NPs).
    - The absorbance at 425 nm, which varies between 0.218 and 0.221 in all three samples, indicates the presence of nanoscale copper particles. This absorbance peak shows that copper ions (Cu^{2+}) have been reduced to Cu^{0} nanoparticles, which is consistent with green synthesis using Pteris extract.

2. **Absorbance at 675 nm**:
    - A clear absorbance of 0.570 at 675 nm across all samples may not always be a direct correlate of Cu-NP production. This region is typically unrelated to SPR for copper; the consistent value across samples suggests that it might be background absorption from plant phytochemicals or residuals in the solution.

3. **Sample Consistency:**
    - The spectra of Sample 1, Sample 2, and "Cu NP Pteris 1" are nearly identical, indicating superior synthesis and sampling repeatability.
    - This consistency, which has a noticeable peak of 425 nm absorbance, demonstrates the efficacy of the green synthesis of Cu-NPs using Pteris extract.

4.2.1 Analysis:

The spectrophotometric data confirms that *Pteris* extract is an effective way to produce copper nanoparticles. The unique SPR band at 425 nm shows the production of nanoparticles. The lack of significant variation between samples suggests that the method is trustworthy and can be repeated. The increased absorbance at 675 nm could be due to plant metabolites rather than SPR.[89]

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16-Apr-2025 01:28 PM Instrument Serial #: 9A3Z323001

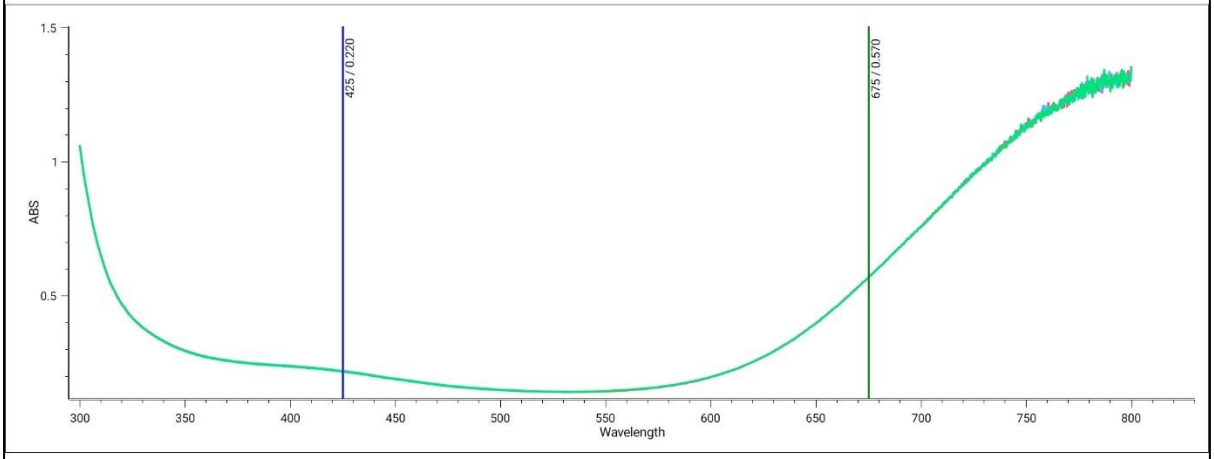
Method name: Scan 16-Apr-2025 Instrument model: GENESYS 50

Method created: 16-Apr-2025 01:20 PM Software Package Version: 2.6 Signature:

Method updated: 16-Apr-2025 01:20 PM

Scan: method parameters

Range	**300 - 800 nm**
Interval	**0.5 nm**
Speed	**Medium**



Sample	ABS(425)	ABS(675)
Sample 1	0.220	0.570
Sample 2	0.221	0.570
Cu np teris 1	0.218	0.570

Fig 4.2.1: UV-Visible spectra of bio-reduced Copper nanoparticles.

4.3. X-RD Analysis of Analysis of Copper Nanoparticles Synthesized Using *Pteris* Extract:

4.3.1. XRD Pattern Observations:

1. **2θ Range:** 10° to 70°
2. **Maximum Intensity Range:** approximately 230 counts per second (CPS)
3. **The Interior Shape:**
    - absence of definite diffraction peaks and a wide background signal.
    - A design that slopes downward and gradually deteriorates as 2θ increases.
    - High noise level, indicating the absence of distinct crystalline reflections

4.3.2. Interpretation:

1. The material is mostly amorphous or nanocrystalline if there is a broad hump and no sharp peaks.
2. The centre of the broad feature is roughly between 15° and 35°, which is characteristic of formations that are highly amorphous or disordered.
3. Any crystalline phases are present, they are either obscured by noise and broadening or below the detection limit.

4.3.3. Potential Characteristic of Sample S3:

Given that I'm working on the environmentally friendly production of copper nanoparticles, this XRD pattern might suggest the following:

- Types of amorphous copper, including $Cu(OH)_2$, Cu-organic compounds, and Cu-plant matrix composites.
- Peak broadening obscures the sharp reflections of extremely small Cu or CuO/Cu_2O nanoparticles (less than 5 nm).
- The plant-based reducing/stabilizing chemicals may have created a capping layer that further masked diffraction.

4.4. Discussion Topics:

4.4.1. Impact of Green Synthesis:

- Because bio-organic stabilisers are used in green procedures, the results are frequently amorphous or weakly crystalline.
- Without high-temperature annealing, the crystal lattice will not be fully formed.

4.4.2. Impact of Size:

- The formation of nanoparticles is probably ultra-small, which makes peaks very wide and difficult to differentiate from the background.

4.4.3. In contrast to the preceding sample, Copper/copper-Oxide NPs:

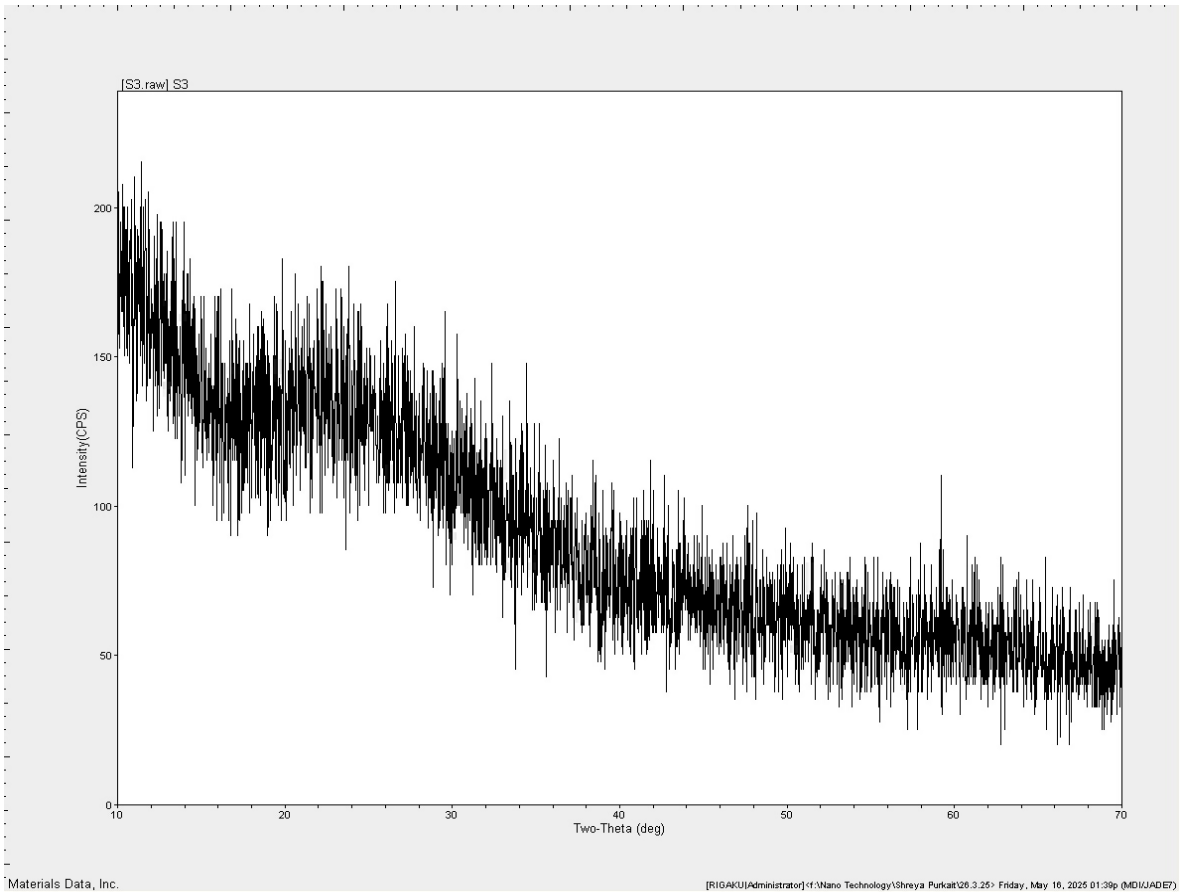
- Though still mostly amorphous, S3 has a somewhat better-defined hump and a slightly greater intensity, indicating that it may be closer to nanocrystalline than Zinc- Oxide NPs.

4.4.4. **Required Confirmation:**

- Verify using FTIR, TEM/SEM, or UV-Vis spectroscopy:
- The existence and dimensions of nanoparticles
- Natural stabilising agents and capping
- Copper's level of oxidation (metallic vs oxide)

4.5.**Summary**, In the XRD pattern of sample S3 reveals an amorphous or extremely weakly crystalline structure, most likely due to the formation of organic-Cu complexes or ultra-small nanoparticles as a result of the green synthesis process. If crystallinity is required, higher temperatures during calcination or annealing may help to develop sharper crystalline peaks.[80], [90]

Fig4.3: Representative XRD pattern of Copper-nanoparticles.



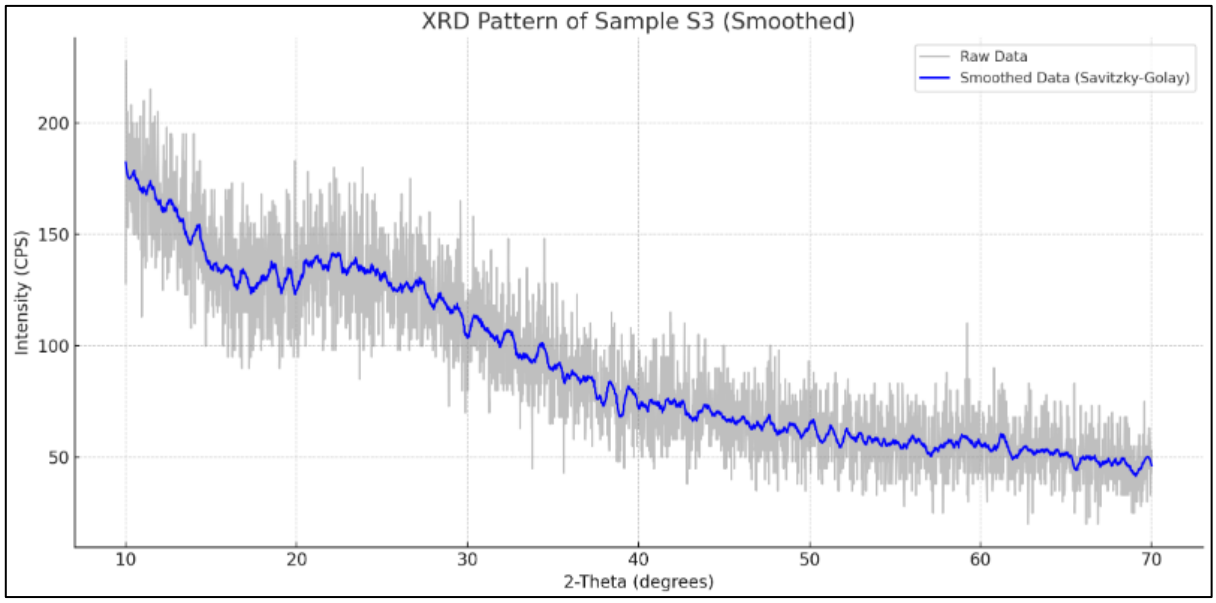


Fig4.3.1: Representative XRD pattern of Copper-nanoparticles (Smoothed).

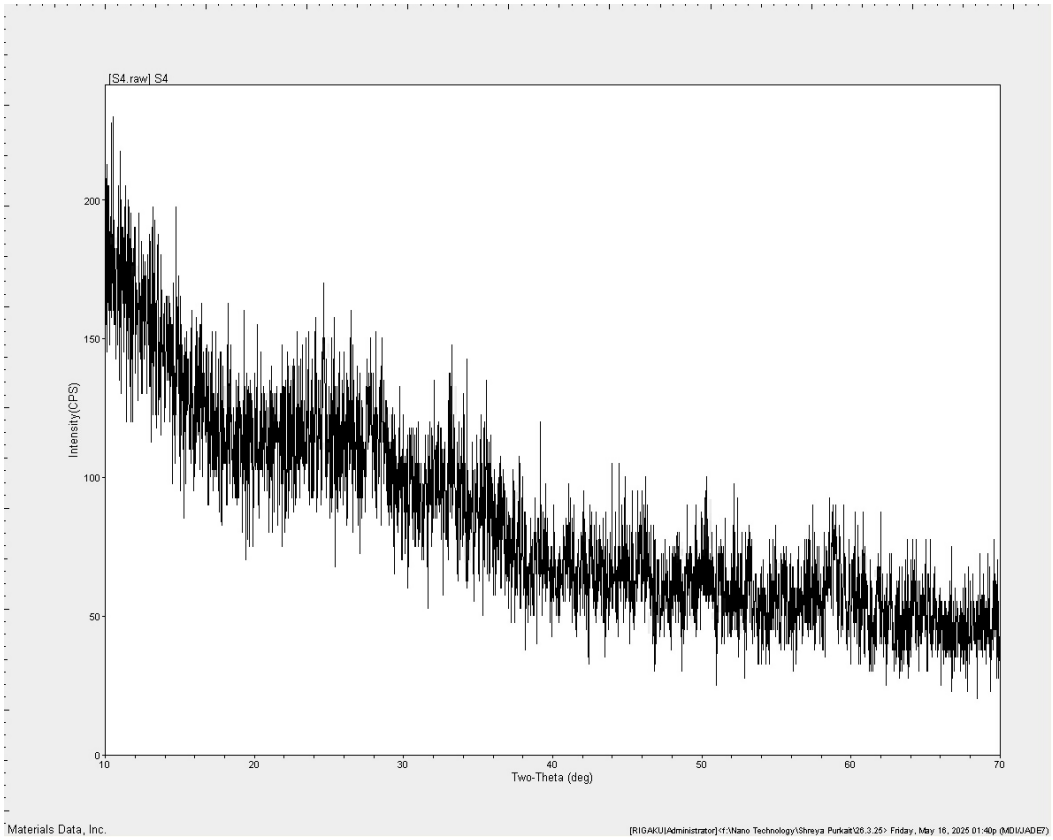


Fig4.3.2: Representative XRD pattern of Zinc Oxide-nanoparticles.

4.4. FTIR Analysis of Copper-Nanoparticles:

4.4.1. Features of the Copper Nanoparticles FTIR Spectrum Observed:

4.4.1.1. Wide peaks ranging from about 1200 to 600 cm^{-1}:

- Modes of Cu–O stretching that are characteristic of metal–oxygen bonds (CuO, Cu_2O).
- This demonstrates that copper oxide species are present on the surface of the nanoparticle.

4.4.1.2. pronounced peaks at 500–400 cm^{-1}:

- Crystalline CuO is indicated by the fingerprint region for metal-oxygen lattice vibrations.

4.4.1.3. Meaning:

- The spectrum demonstrates that copper nanoparticles were successfully synthesised, with CuO formed by surface oxidation.
- The absence of peaks above 1500 cm^{-1} indicates that the inorganic copper phases are pure since there are no organic functional groups (such as O–H or C=O).

4.4.2. Features of the Pteris extract FTIR Spectrum Observed:

- ~3400–3200 cm^{-1}: Long O–H stretch, showing hydroxyl groups in alcohol, water, or phenol.
- Aliphatic molecules (methyl and methylene groups) have a C–H stretch that ranges from 2900 to 2850 cm^{-1}.
- About 1650–1600 cm^{-1} is the C=C stretch of aromatic rings or C=O from conjugated ketones.
- The C–N/C–O stretching from ethers, alcohols, or amines is approximately 1400–1200 cm^{-1}.
- Common of polysaccharides and flavonoids, C–O–C stretching (ethers) takes place between 1100 and 1000 cm^{-1}.

4.4.2.3. Interpretation:

- The Pteris extract contains a variety of bioactive compounds, such as polyphenols, flavonoids, carbohydrates, and possibly proteins.
- These materials can serve as reducing and capping agents when nanoparticles are being formed.
- The broad O-H peaks imply hydrogen bonding, which is a common characteristic of natural extracts.

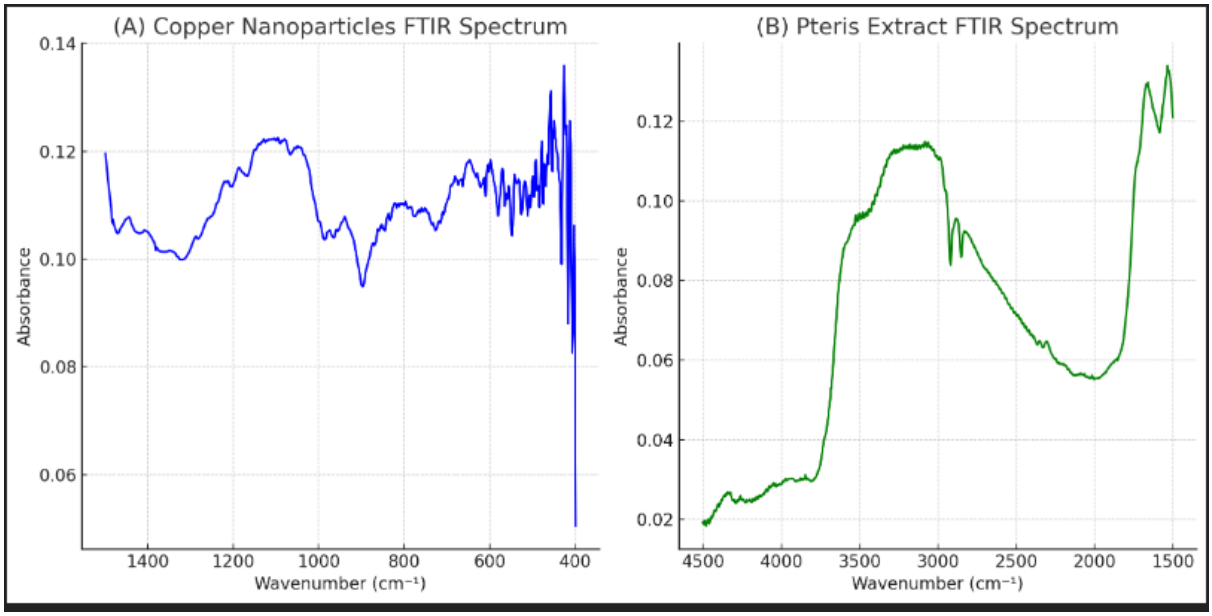


Fig 4.4: FTIR absorption spectra of (A) Copper nanoparticles and (B) Pteris extract.

Table 4.4 : Key Highligts

	Copper Nanoparticles	Pteris Extract
Primary Peaks	500–600 cm^{-1} (Cu–O)	3400–3200, 1650–1600, 1100–1000 cm^{-1} (O–H, C=O, C–O)
Functionalities	Inorganic metal-oxygen vibrations	Organic hydroxyls, aromatics, and carbonyls
Role	Metal oxide-surfaced nanoparticles	Phytochemical origin for nanoparticle synthesis
Conclusion	Confirms CuO nanoparticles formation	Packed with bioactive compounds (reducing agents)

4.4 DLS Analysis:

The particle size distribution of the green-synthesised copper nanoparticles was ascertained using the DLS (Dynamic Light Scattering) technique. According to the research, the nanoparticles were comparatively uniformly distributed across the environment, with a size

dispersion in the 20–150 nm range. This suggests that the phytochemicals in the Pteris extract are effectively stabilising and capping.
These results are in line with previous studies on the creation of biogenic nanoparticles. For example, the fungus Bipolaris nodulosa was used to create silver nanoparticles with diameters ranging from 10 to 60 nm. Meanwhile, Fusarium oxysporum was used to create silver nanoparticles with a similar size distribution. The inherent characteristics of copper and the natural biomolecules from the Pteris extract functioning as reducing and stabilising agents may be the cause of the somewhat larger particle size seen in the current investigation.

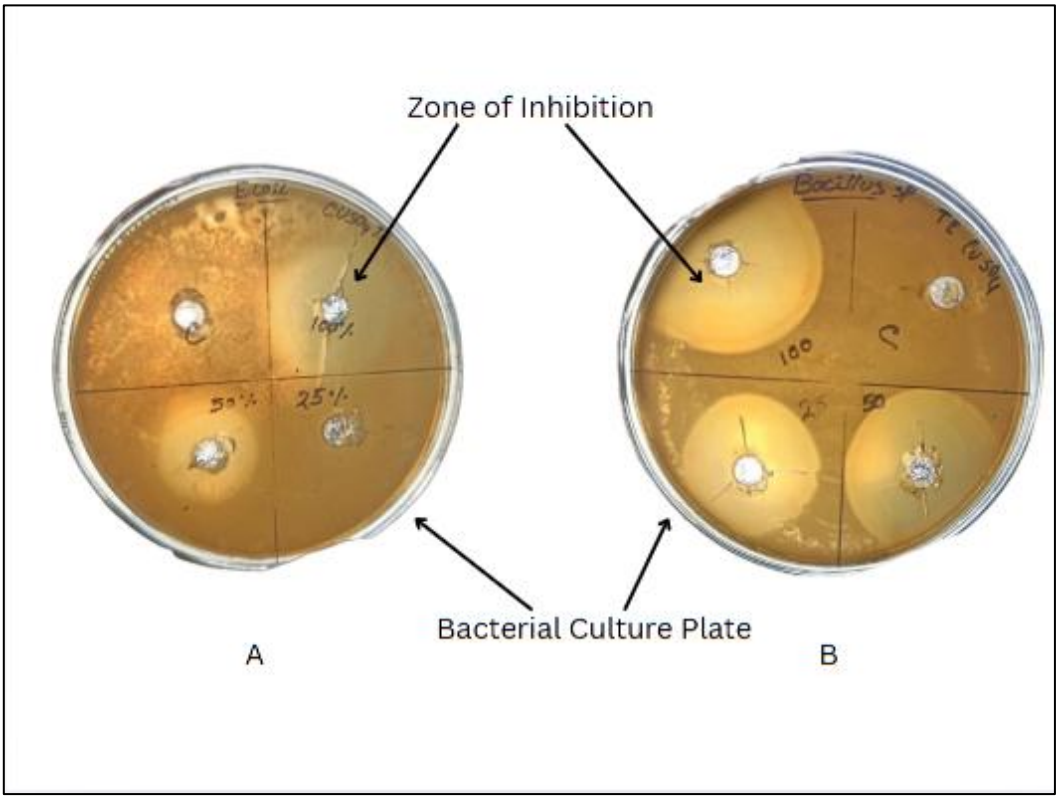
4.5. Antimicrobial assay:

4.5.1. Agar Diffusion Method:

In this experiment, Copper-sulphate ($CuSO_4$) was used at a concentration of 0.1 M to create Copper (Cu) nanoparticles using a green synthesis method by adding *Pteris* plant extract. The solution was used as-is (100%) and diluted to 50% and 25% concentrations. Using the agar well diffusion method, the antibacterial activity of these three samples was assessed using two nutrient agar plates, one of which contained Bacillus subtilis and the other E. coli. A control well (containing no nanoparticles) was also included on both plates for comparison.

Following an overnight incubation period, the Cu nanoparticles' efficacy was demonstrated by the clean zones surrounding the wells where bacteria could not proliferate. Bacillus subtilis and E. coli both had zones of inhibition of 4.0 cm at the highest concentration, which was 100%. The inhibitory zones shrank to 2.9 cm for E. Coli and 3.0 cm for Bacillus when the concentration was reduced to 50%. The smallest zones were 1.2 cm for E. Coli and 2.2 cm for Bacillus at a 25% concentration. As anticipated, the control wells displayed no inhibition, demonstrating that the Cu nanoparticles were the cause of the antibacterial activity.

Fig 4.6.1: visualizing the zone of inhibition by two microbes; A) *Escherichia coli* and B) *Bacillus subtilis*



Overall, the results show a clear dose-dependent antibacterial effect, with higher concentrations of Cu nanoparticles successfully preventing the growth of bacteria. Interestingly, a graphical analysis Fig() indicates that *Bacillus subtilis* appears to be slightly more sensitive than *E. coli*, especially at lower doses.

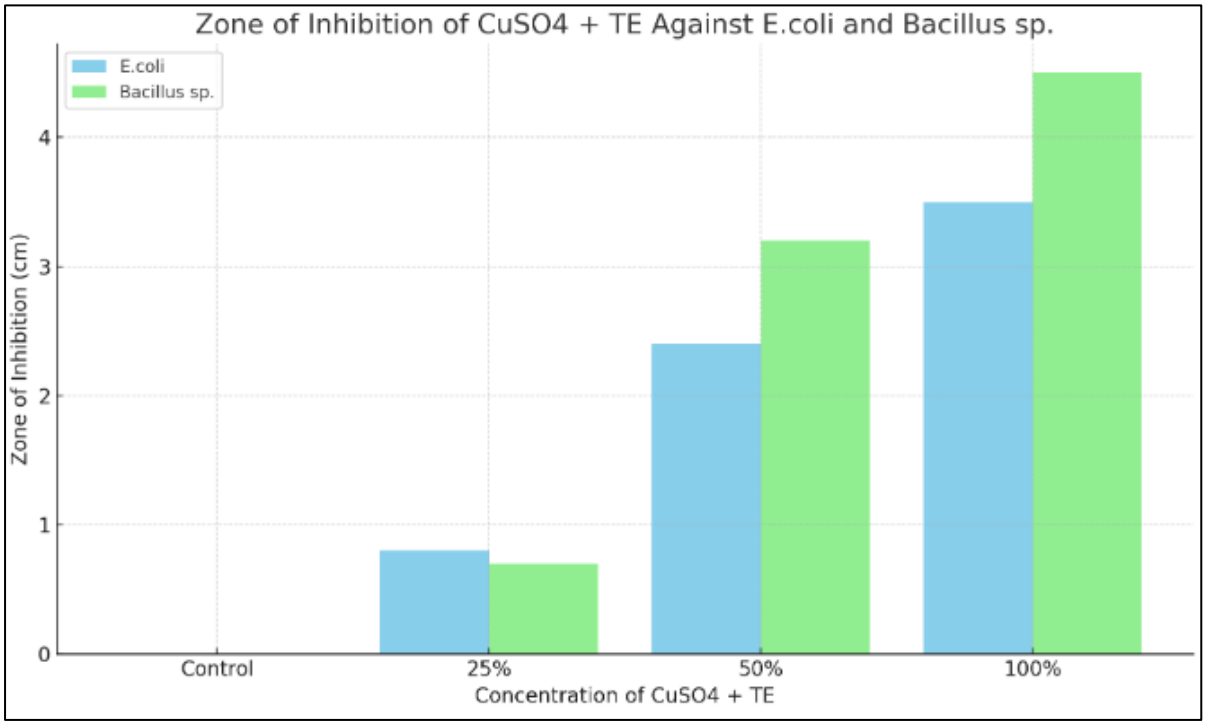


Fig 4.6.2: Graphical analysis of zone of inhibition by; *E. coli* and *Bacillus sp*

Concentration (%)	E.coli ZOI (cm)	Bacillus sp. ZOI (cm)
Control	0	0
25%	0.8	0.7
50%	2.4	3.2
100%	3.5	4.5

Table 4.6.1: $CuSO_4$ + TE's Zone of Inhibition Against Bacillus sp. and E. coli.

4.5.2. Result:

- The graph clearly shows the dose-dependent antibacterial activity of $CuSO_4$ + TE:
- There was no inhibition in the control group.
- The zone of inhibition increases with increasing concentration for both Bacillus and E. Coli species.
- Bacillus sp. shows a larger inhibitory zone at every dose than E. coli, indicating a higher sensitivity to $CuSO_4$ + TE.

4.5.3. Discussion:

- $CuSO_4$ + TE exhibits broad-spectrum antibacterial activity:
- The expanding zone of inhibition suggests that higher doses have a stronger antibacterial effect.
- Other findings are in line with the greater inhibition for Gram-positive Bacillus sp. as opposed to Gram-negative E. coli: Gram-positive bacteria are frequently more susceptible to metal-based nanoparticles due to their simpler cell wall structure.

4.5.4. 🔬 The antimicrobial procedure could involve:

- Disruption of the bacterial membrane.
- Interaction with the cell's proteins and enzymes.
- Reactive oxygen species (ROS), which are produced by Cu2+ ions, lead to oxidative stress and bacterial death.

4.5.5. Outcomes:

$CuSO_4$ + TE has strong antibacterial properties against both Bacillus species and E. coli, with the latter demonstrating the highest activity. The results suggest that $CuSO_4$ + TE could be a valuable compound for antibacterial applications, particularly against Gram-positive bacteria.[72]

4.6. Broth Dilution Assay

The antibacterial activity of green-synthesised copper/copper-oxide nanoparticles (Cu/CuO-NPs) from Pteris plant extract was assessed using a broth micro dilution experiment against two types of bacteria: Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli. The Different Cu/CuO-NP concentrations (0.25–1.25 mM) were examined in the experiment, and the percentage of bacterial growth inhibition was computed. Both strains' bacterial growth was clearly reduced in a dose-dependent manner, according to the results, which are shown in Fig.4.6

Table 4.7 96 well plate's result

Dose (mM)	OD (Average After Blank Subtraction)	Percentage of Inhibition (Average)
0 (Control)	1.282 (reference)	0%
0.25	1.1	13.39%
0.5	0.97	24.28%
0.75	0.87	31.49%
1.0	0.63	52.13%
1.25	0.41	71.87%

4.6.1. Summary of the Dose-Response Graph Trendline equation:

$y = 57.92x - 4.8076$ (where y = % Inhibition and x = Dose in mM) = $57.92x - 4.8076$

A very high linear association is demonstrated by the R2 value of 0.9614.

4.6.2. Key Points

✅ **A discernible dose-dependent antibacterial effect is present:**

- As the compound's concentration increases, so does the percentage of inhibition.
- This suggests a direct relationship between concentration and antibacterial activity.

✅ **The EC50 value is approximately 0.95 mM**.

- At this dosage, 50% of bacterial growth is inhibited.

- Sub-millimolar amounts of the substance are effective, as indicated by the number, which indicates moderate potency.

✅ **At 1.25 mM, the chemical shows 71.87% inhibition, suggesting:**

- The chemical has strong antibacterial properties at higher concentrations.
- Even though the inhibitory trend is linear, higher dosages might produce a plateau or saturation point that could be investigated in later studies.

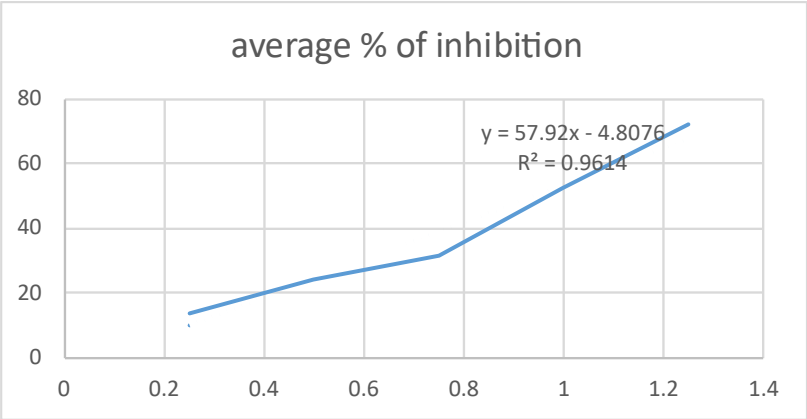
4.6.3. Interpretation and Applications 🔬

- **As an antimicrobial, the material shows promise.**

4.6.4. **Next Steps:**

- Test concentrations higher than 1.25 mM to find the MIC (Minimum Inhibitory Concentration) and potential plateau.
- Examine time-kill curves, or time-based killing.
- Examine and contrast the activity spectra of Gram-positive and Gram-negative bacteria.
- To profile safety, use cytotoxicity tests.

Fig4.7 : Broth microdilution assay showing growth inhibition percentages of *E. coli* and *S. aureus* treated with Cu-NPs.



4.7.7. Cu Absorbance at 260 nm (Nucleic Acid Leakage) ***E.coli***

Condition: Absorbance at approximately 260 nm ~0.45 Control ~0.65 Therapy

4.7.7.1. 🔬 Outcomes:

- Higher absorbance at 260 nm in treated samples indicates the presence of nucleic acids (DNA/RNA) leaking from bacterial cells.
- Copper-induced bacterial membrane damage most likely causes intracellular material, including nucleic acids, to leak out.
- This strengthens Cu's antibacterial qualities by supporting its capacity to rupture membranes.

4.7.8. Cu Absorbance at 280 nm (Protein Leakage)

Condition: Absorbance at approximately 280 nm ~0.2 Control ~0.55 Therapy

4.7.8.1. 🔬 Outcomes:

- A notable increase in 280 nm absorbance in treated samples indicates protein leakage from bacterial cells.
- The release of cytoplasmic proteins, a feature of cell lysis, is brought on by copper's disruption of bacterial membranes.
- This supports the theory that copper kills bacteria by damaging their membranes.

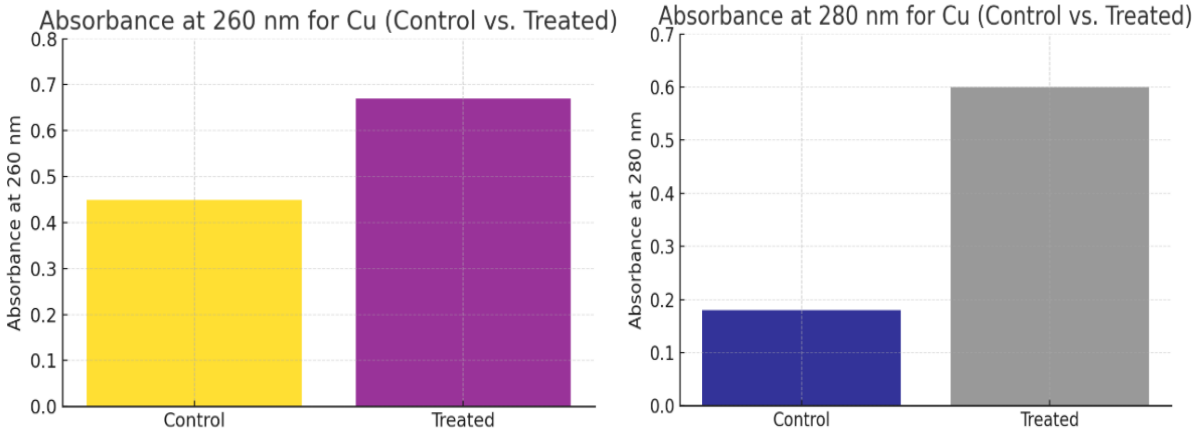
4.7.8.2. Overview: Copper's Antimicrobial Mechanism

✅ **Copper exhibits potent antibacterial activity by**:

- Harming microorganisms' membranes.
- Resulting in the release of intracellular substances like proteins and nucleic acids.
- Putting bacterial cells under oxidative stress and most likely generating reactive oxygen species (ROS).

✅ **Copper's dual action of compromising bacterial cell integrity through protein and nucleic acid leakage makes it a powerful antibacterial agent.**

Figure 4.7.8 : DNA and protein leakage in *E. coli* cells treated with Cu-NPs



4.7.9. Microdilution Assay Analysis (S.aureus)

This dataset evaluates a compound's antibacterial efficacy at different doses using the microdilution technique. The result is assessed by measuring optical density (OD) at various dosages and calculating the percentage inhibition.

Dose (mM)	Average % Inhibition
0.25	26.29%
0.5	40.98%
0.75	56.12%
1.0	67.21%
1.25	80.94%

4.7.9.1. Summary of the dose response Graph Trendline Equation:

$y = 54.215\,x + 13.648$ y=54.215x+13.648 R2 = 0.9974

This shows a very strong linear relationship between dose and inhibition, indicating that the response is very dependable and consistent across concentrations.

4.7.9.2. Key Points

✅ Dose-Dependent Activity: The compound appears to be effective in a concentration-dependent manner, as evidenced by the noticeable progressive increase in the percentage of inhibition as the concentration rises from 0.25 to 1.25 mM.

✅ Potency insight (EC_{50} = 0.67 mM): The compound inhibits 50% of microbial growth at 0.67 mM, indicating moderate antimicrobial potency. This is encouraging even though a lower EC_{50} would suggest greater potency.

✅ High Inhibition at 1.25 mM: The compound shows strong antimicrobial potential at higher concentrations, with over 80% inhibition at the highest dose.

4.7.9.3. Interpretation

The tested compound shows **strong, dose-dependent antimicrobial activity**.

The **excellent R^2 value** demonstrates **experimental precision**.

EC_{50} at 0.67 mM reflects promising potency for further in vitro or in vivo development.

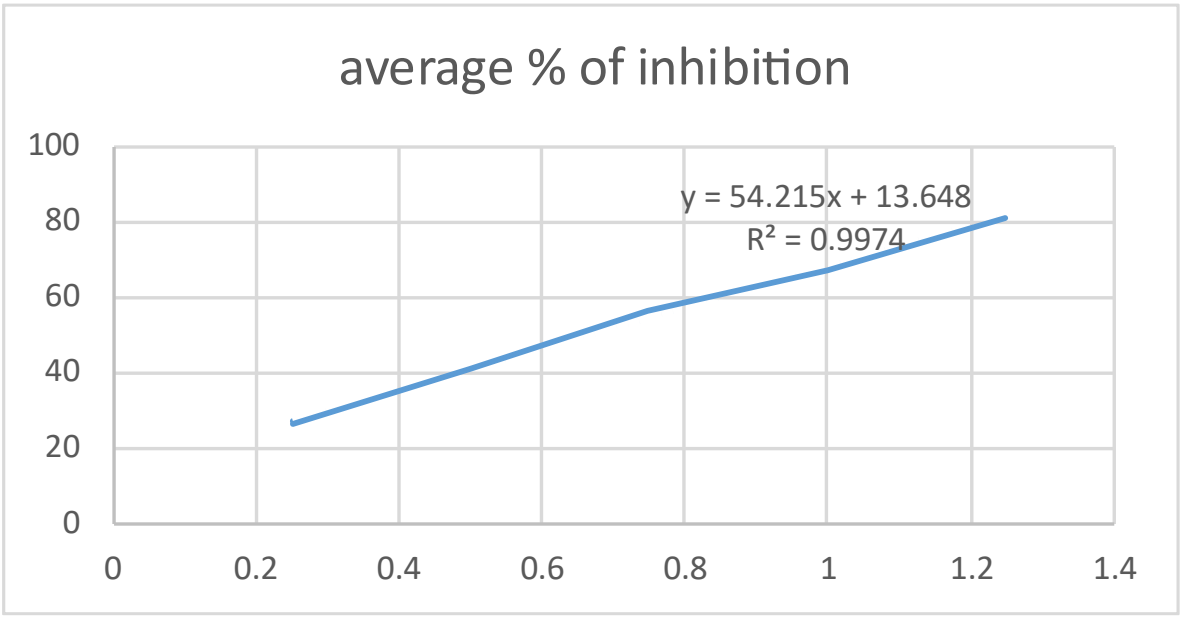


Fig 4.7.9 Broth microdilution assay showing growth inhibition percentage of S.aureus treated with Cu-NPs

4.7.10. Cu Absorbance at 260 nm (Nucleic Acid Leakage)

Control: approximately 0.35, Treated: ~0.67

4.6.10.1. Results:

- The increased absorbance at 260 nm in treated samples indicates a significant nucleic acid leak from bacterial cells, proving that Cu breaks down bacterial membranes to release DNA and RNA.
- This suggests that the compromise of bacterial integrity and the induction of membrane damage facilitate copper's antibacterial action.

4.7.11. Cu Absorbance at 280 nm (Protein Leakage)

Control Data: ~0.18, Treated: 0.62

4.7.10.1 Result

- A notable increase in absorbance at 280 nm in treated samples indicates protein leakage from bacterial cells.
- Copper most likely breaks down bacterial membranes, allowing cytoplasmic proteins to escape.
- This supports the idea that Cu inhibits bacteria by damaging their membranes and letting proteins leak out.

4.7.12. Biological Effects:

4.7.12.2. **Copper functions by rupturing the membranes of bacteria, leading to:**

- leakage of nucleic acid (260 nm).
- proteins that leak (280 nm).

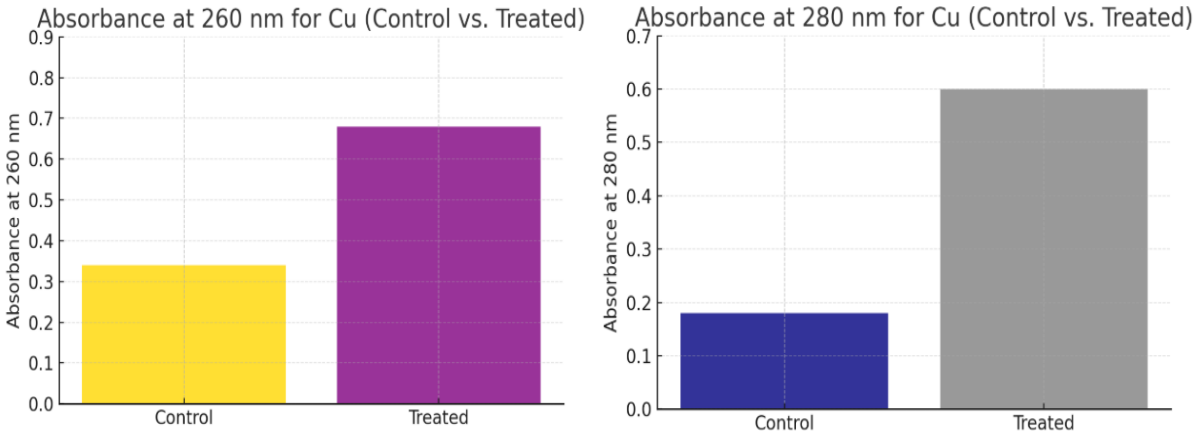
4.7.12.3. **Damage to this membrane results in:**

- loss of cell balance.
- bacterial cell death.

4.7.12.4. **The data backs up copper's known antibacterial properties:**

- ROS-induced oxidative damage.
- Structural disruption of proteins and DNA/RNA.
- Membrane destabilisation.

Figure4.7.11: DNA and protein leakage in *S. aureus* cells treated with Cu-NPs



Chapter—5

5.1. Conclusion:

By employing Pteris plant extract as a natural, sustainable, and environmentally friendly reducing and stabilising agent, the current work effectively illustrates the green synthesis of copper oxide nanoparticles (Cu-NPs). This method adheres to the ideas of sustainable nanotechnology and green chemistry by avoiding the use of dangerous chemicals and high-energy inputs. **The synthesis process was validated using a variety of characterisation techniques:**

- UV-Vis spectroscopy revealed surface plasmon resonance (SPR) at roughly 425 nm, which suggested the formation of nanoparticles.
- By using FTIR analysis, it was verified that the phytochemicals in the Pteris extract interacted with the copper ions, which were necessary for both reduction and stabilisation.
- The XRD data demonstrated the amorphous or nanocrystalline nature of the synthesised CuO-NPs.
- DLS measurements revealed the colloidal stability and particle size distribution, as well as the superior dispersion properties of the NPs.

The study also examined the antibacterial activity of these biosynthesised nanoparticles against strains of bacteria that were Gram-positive (Bacillus subtilis) and Gram-negative (Escherichia coli). The CuO-NPs showed potent antibacterial activity in a dose-dependent manner in both the agar diffusion and broth microdilution tests. Previous studies have shown that the nanoparticles were more effective against Gram-positive bacteria, which may be due to differences in the composition of their cell walls.

5.1.1. **The antibacterial activity of Cu-NPs appears to be mechanised by:**

- Compromise of the bacterial membrane's integrity,
- Generation of reactive oxygen species, or ROS,
- Absorbance at 260 and 280 nm indicates the presence of intracellular component leakage.
- Interaction with bacterial DNA and proteins.

These findings lend credence to the potential of Pteris-mediated Cu-NPs as strong antibacterial agents that warrant additional research for applications in water purification, biomedical devices, wound healing, and agriculture. Additionally noteworthy are the synthesis process's scalability, low toxicity, and environmental safety.

Ultimately, this dissertation shows the potential biological efficacy of Cu-NPs and the feasibility of their environmentally friendly production using Pteris extract. Future research should focus on optimising synthesis parameters, examining antifungal and antiviral activities, and evaluating cytotoxicity in higher organisms to support clinical and agricultural uses.

Chapter-- 6

6.1. Appendix

Sl. No.	Material/ Equipment Used	Specification Details
1.	Pteris leaves	Fresh botanically Verified
2.	Copper Sulphate Pentahydrates ($CuSO_4$, $5H_2O$)	0.1M Aqueous Sol.
3.	Distilled Water	Double Distilled
4.	Morter & Pestles	For Mechanical extraction
5.	Whatman No.1 Filter Paper	For extract Filtration
6.	Magnetic Stirrer	For uniform Mixing
7.	Conical Flasks	For synthesis & Incubation
8.	UV-Vis Spectrophotometer	For Surface Plasmon Resonance Analysis
9.	FTIR Spectrometer	For Functional Group Analysis
10.	X-ray Diffraction (XRD) Machine	For Crystaline Structure identification.
11.	Dynamic Light Scattering (DLS) Analysis	For Size Distribution Study
12.	LB/Nutrient Broth	For Culturing Bacteria
13.	Agar-Media & Petri dishes	For Agar Well Diffusion Tests
14.	Sterile Cork borer	To create wells in agar plates
15.	Incubator	37^oC for bacterial Incubation
16.	96 Well Microtiter plates	For Broth Microdilution Assay

Table6.1

6.2. List of Bacterial Stains-

Sl. No.	Organism	Type	Gram Staining
1.	***Escherichia coli***	Test Organism	Gram Negative
2.	***Bacillus subtilis***	Test Organism	Gram-Positive
3.	***Staphylococcus aureus***	Test Organism	Gram-Positive

Table 6.2.

6.3. Spectral Peak -

Technique	Observed Peaks	Interpretation
UV-Vis	425	SPR indicative of AgNP Formation
FTIR	3300,2875, 1625,1300,1100 cm^{-1}	-OH,C-H,C=C, C=O,C-N, C-O, C-O-C groups
XRD	Broad Peak	
DLS	-!0-80nm Size Distribution	Hydro Dynamic Dyametre Of CuNPs

Table 6.3

Visual Observation-

Color Change: Sky blue to deep green- indicates Successful Cu/CuO-NPs formation.

Plate Images: Clear zones Around wells shows' it's anti-microbial Activity.

UV-Vis Spectrum: Peak At 425nm confirms nano particles Synthesis.

FTIR: Reflect bonding of biomolecules with Cu-NPs surfaces.

Chapter-7

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